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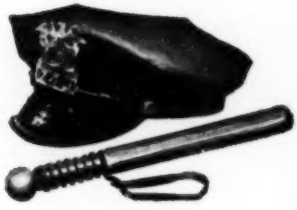

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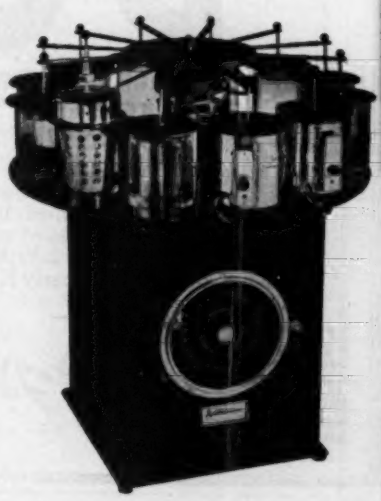

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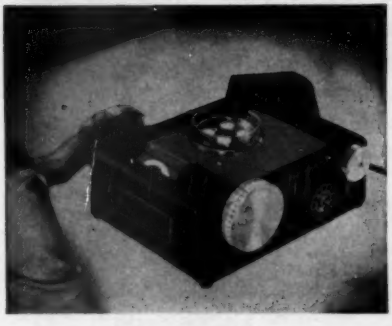

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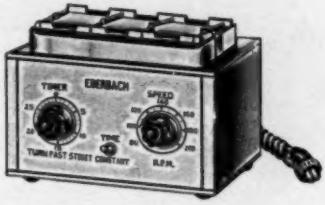

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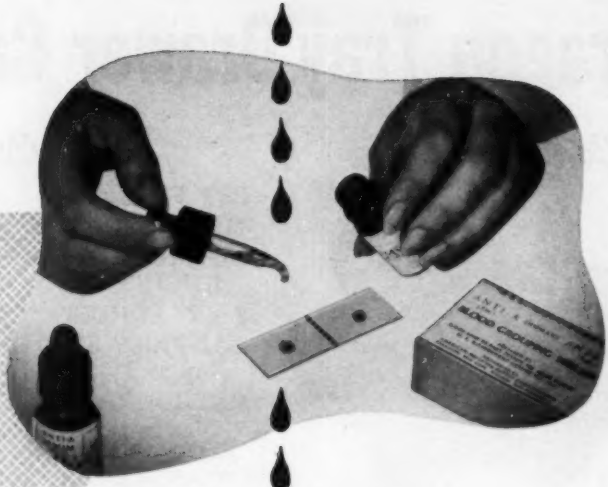

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A STUDY OF TUMOR TISSUE AFTER TRANSPLANTATION

Histologic Changes Observed in Anterior-Chamber Transplants of Heterologous Tumor Tissue

ABE TOWBIN, M.D.
COLUMBUS, OHIO

HETEROLOGOUS tumor tissue transplanted to the anterior chamber of the eye provides a unique means of studying the changes that occur in tumor tissue after transplantation. In the past, the use of subcutaneous homologous tumor transplants and other methods of investigating this problem has met with many limitations. The consistent recovery of small subcutaneous transplants proves difficult. Studies correlating progressive gross and microscopic changes cannot be readily carried out on transplants covered by skin. When tumor tissue is transplanted into subcutaneous pockets, the inflammatory reaction resulting from the operative procedure complicates the study of early local tissue changes.[1a]

In the present study, in which the transplants were cultivated in the anterior chamber of the eye, it was possible to observe directly the successive changes in the gross appearance of the tumor tissue through the transparent cornea. With this technique the transplant is lodged at a significant distance from the operative site; thus early tissue changes, uncomplicated by operative inflammation, may be studied. At selected stages of development the transplanted tissue can be readily recovered for histologic examination. The iris and other structures of the eye provide well-defined landmarks so that even small deposits of transplanted tissue can be distinguished from host tissue.

If homologous tumor tissue is used, the rapid development and growth of the transplant prevent accurate definition of early tissue changes; if, however, heterologous tumor tissue is substituted, the succession of tissue changes is retarded. Studies in the past have indicated that transplantation of heterologous tumor tissue is governed to a large degree by the species difference between the donor animal and the new host.[1b] As the species barrier is increased, transplantability of tumor tissue is impeded. By selecting donor and recipient animals of appropriate species difference the progression of changes at the site of transplantation may be slowed and the details of each phase can be demonstrated. In short, such a technique provides a "slow motion" projection of an otherwise rapid succession of tissue changes.

From the Department of Pathology, Ohio State University College of Medicine.

This project was supported by grants from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

1. Loeb, L.: (*a*) The Biological Basis of Individuality, ed. 1, Springfield, Ill., Charles C Thomas, Publisher, 1947, p. 30; (*b*) p. 341.

The anterior-chamber technique provides a natural milieu for the development and growth of the tumor transplants.[2] The development and use of this technique have been reviewed recently by Morris, McDonald, and Mann.[3] By this method direct observation of the transplant is possible without the necessity of using a transparent covering material of a foreign nature at the site of transplantation.

MATERIAL AND METHOD

The present study is based on observations made during the course of determinations of tumor growth carried on in this laboratory; heterotransplantation of 38 animal tumors and 100 different human neoplasms has been completed to date; an analysis of the results of these series of transplantations forms the basis of a separate report.[2]

The animal tumors, representing a gradient of species, including mouse, cat, dog, cow, and horse, were transplanted in order to observe the effect of species difference on transplantability

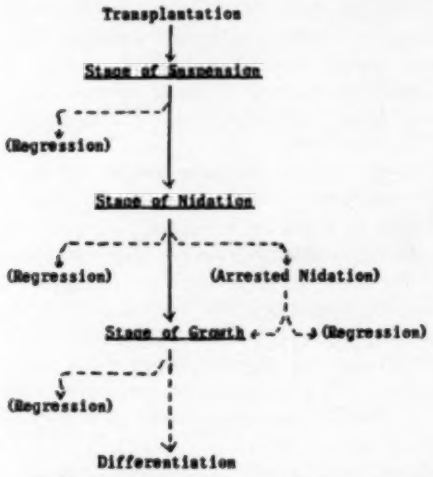

Fig. 1.—The establishment and growth of tumor transplants.

of neoplasms. In most experiments the guinea pig was used as the host animal. Studies by Greene[4] have indicated that heterologous transplantation of tumors is more often successful with the guinea pig than with other laboratory animals.

Before transplantation of tumor tissues the guinea pigs were anesthetized with pentobarbital sodium intraperitoneally. Under sterile conditions the tumor tissue was introduced into the anterior chamber of the eye with a no. 16 trochar through a small incision in the upper sclerocorneal junction. The transplant was then maneuvered away from the incisional area into the lower angle of the chamber by stroking the outer surface of the cornea with a blunt probe. After transplantation, progressive macroscopic changes in the tumor tissue were studied

2. Towbin, A.: Heterologous Transplantation of Human Tumors, Cancer Res., to be published.

3. Morris, D. S.; McDonald, J. R., and Mann, F. C.: Intraocular Transplantation of Heterologous Tissues, Cancer Res. **10**:36, 1950.

4. Greene, H. S. N., and Lund, P. K.: The Heterologous Transplantation of Human Cancer, Cancer Res. **4**:352, 1944.

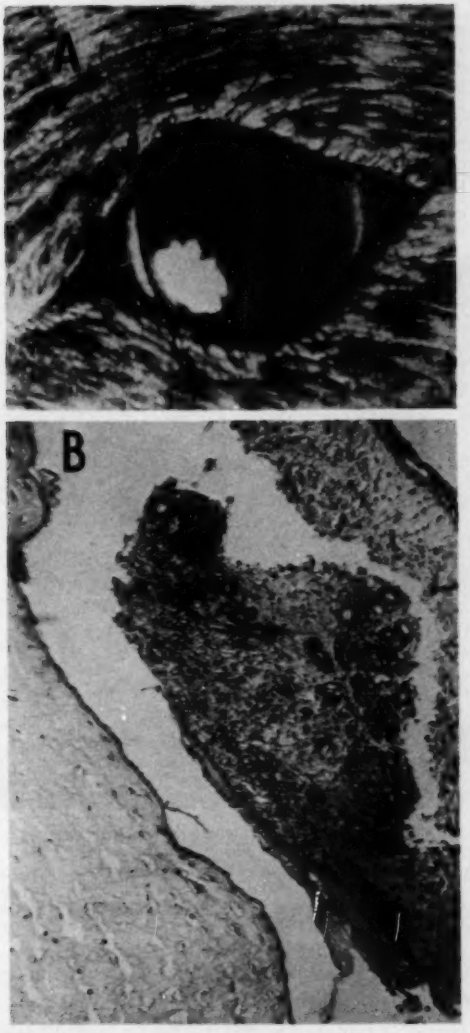

Fig. 2.—Suspension stage: *A*, gross appearance of a tumor transplant in the anterior chamber of the guinea-pig eye directly after transplantation. × 6. *B*, carcinoma of the breast (C3H mouse) 48 hr. after it had been transplanted to the anterior chamber of a guinea pig's eye. The transplant, showing well preserved marginal cells and a necrotic central area, lies loosely between the iris and the cornea. × 70.

in situ through the cornea; at selected intervals tumors were harvested for correlation studies of gross and microscopic changes.

EXPERIMENTAL OBSERVATIONS

Three fundamental phases are evident in the process that occurs at the site of transplantation. The pattern of changes is shown in Figure 1. In the first phase temporary sustenance is provided for the tumor tissue by the aqueous humor as the transplant lies free in the anterior chamber; this period may be termed the suspension stage. In the second phase the tumor tissue becomes closely associated with the vascular system of the host; the transplant becomes firmly attached; this is the nidation stage. In the third phase expansive proliferation of the organized tumor tissue occurs; this is the growth stage.

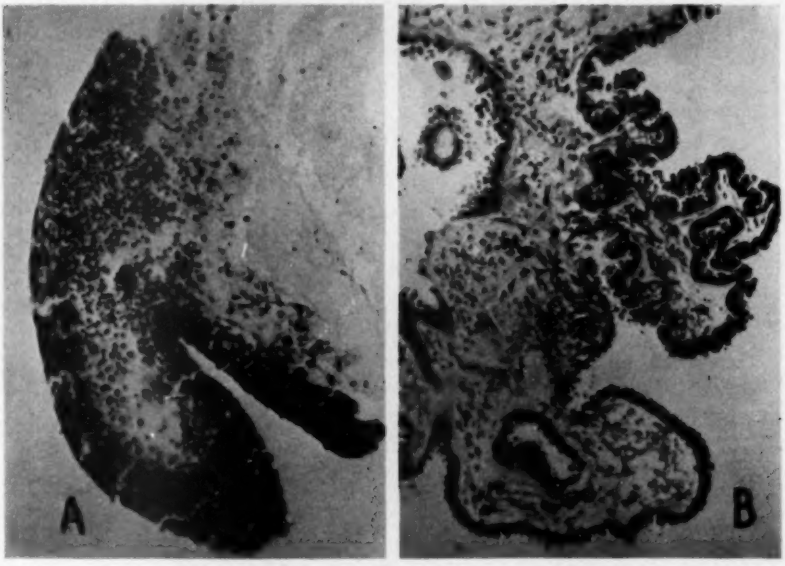

Fig. 3.—Suspension stage, prolonged: *A*, human bronchogenic carcinoma 55 days after it had been transplanted to a guinea pig's eye. The transplant remained loosely suspended in the anterior chamber. The harvested transplant shows preserved marginal tumor cells. × 70. *B*, human papillary cystadenocarcinoma of the ovary 21 days after it had been transplanted to the anterior chamber of a guinea pig's eye. The section from the suspended transplant shows well preserved marginal epithelium. × 120.

The nature of each stage may be resolved by correlating the progressive gross and microscopic changes that occur at the site of transplantation.

Suspension Stage.—The provision of a temporary nutritional medium for the transplant is the effective principle of this stage. During this phase the tumor fragment, lying loosely suspended in the aqueous humor, persists in a "tissue culture" state. The suspension stage usually lasts three to six days. As observed through the cornea the transplant becomes blanched and sharply delineated (Fig. 2*A*).

The histologic features of the suspension stage are demonstrated in Figure 2*B*; this section shows a 48-hr. transplant of an adenocarcinoma of the breast of a C3H mouse in the anterior chamber of a guinea pig's eye. The tumor fragment lies loosely between the iris and the cornea; the central portion of the transplant has undergone necrosis; the integrity of the marginal cells is preserved.

Although some proliferation of a tissue-culture type may occur at this time in the marginal cells, organized growth is not evident until the transplant has evolved through the stage of nidation and has acquired a stromal framework.

Occasionally it was noted that with some transplants the duration of the suspension stage was prolonged. This observation emphasizes that this stage, though usually transient, is a discrete phase.

Figure 3*A* shows a portion of a transplant of a human bronchogenic carcinoma of the small-cell type which remained unattached during a period of 55 days in the anterior chamber; the section shows preserved tumor cells in the marginal zone.

Certain tumors, because of their special structure, appear to be particularly adapted to the free-floating existence of this stage. A verrucose nodule from a human papillary cystadenocarcinoma of the ovary was transplanted to a guinea pig's eye; the tumor fragment remained loosely suspended in the aqueous humor for 21 days. When recovered from the anterior chamber the transplant showed well preserved epithelial elements (Fig. 3*B*).

If in the aqueous humor the nutrition and maintenance of the tumor tissue prove inadequate, the entire transplant undergoes necrosis, and gradual absorption occurs. If viable tumor cells endure, nidation follows.

Nidation Stage.—The most significant development during this stage is the integration that occurs between the transplanted tumor elements and the vascular system of the new host. The tumor tissue is invaded by stromal elements from the host at the site of transplantation. Observed grossly through the cornea, the tumor tissue assumes a pink color; fine vessels arising from the iris permeate the transplant (Fig. 4*A*). Moderate enlargement of the transplant which may become apparent at this time proves on histologic investigation to be due largely to the process of organization.

Figure 4*B* demonstrates the initial histologic changes that occur in the nidation stage; tissue from an adenocarcinoma of the breast of a C3H mouse was transplanted to the anterior chamber of a guinea pig's eye; the section, taken four days after transplantation, shows a sheet of tumor tissue attached to the iris; blood vessels and other stromal elements, as well as pigmentary debris from the iris, permeate the transplant.

Figure 5*A* shows a transplant early in the stage of nidation. The tumor tissue, taken from a bovine carcinoma of the kidney, became loosely attached to the iris. The transplant was recovered on the seventh day. Histologically, the nodule, surrounded by a thin stromal capsule from the host, shows a necrotic core and well preserved marginal layer of neoplastic cells (Fig. 5*B*).

The duration of the stage of nidation is, in general, directly governed by the degree of species difference between the donor and the recipient animal. It was observed during the present study that mouse tumors such as Sarcoma 37, the Earle fibrosarcoma, and the C3H breast carcinoma, pass through the stage of nidation in three to eight days. With tumor transplants of cat, dog, cow, and horse

tumors, nidation varied from 22 to 88 days. With human tumors, a state of arrested nidation lasting up to 114 days was recorded.

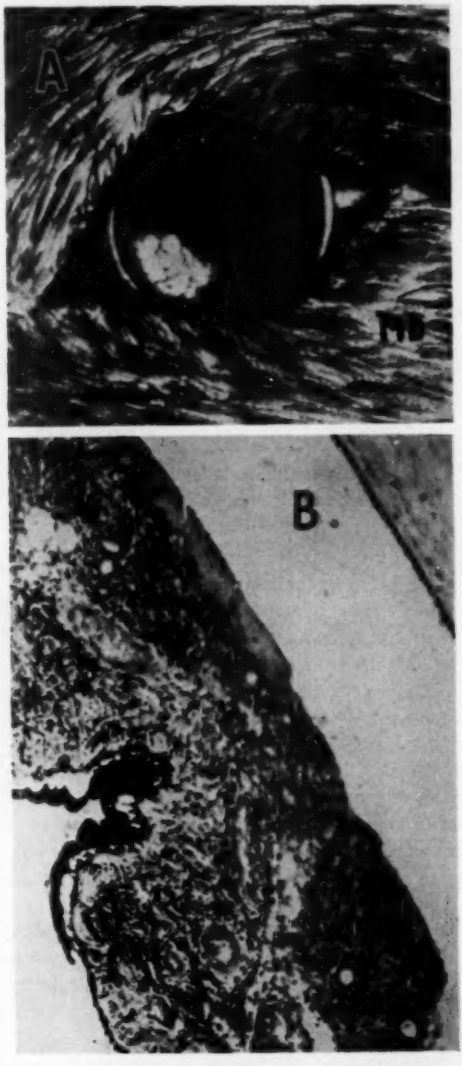

Fig. 4.—Nidation stage: *A*, gross appearance of the vascularized tumor transplant in the anterior chamber of the guinea-pig eye. × 6. *B*, carcinoma of the breast (C3H mouse) four days after it had been transplanted to a guinea pig's eye. The sheet of tumor tissue is attached to the iris; stromal elements from the iris permeate the transplant. × 70.

During nidation the tissue changes that occur are essentially manifestations of an inflammatory reaction. Vascular changes are prominent at the site of trans-

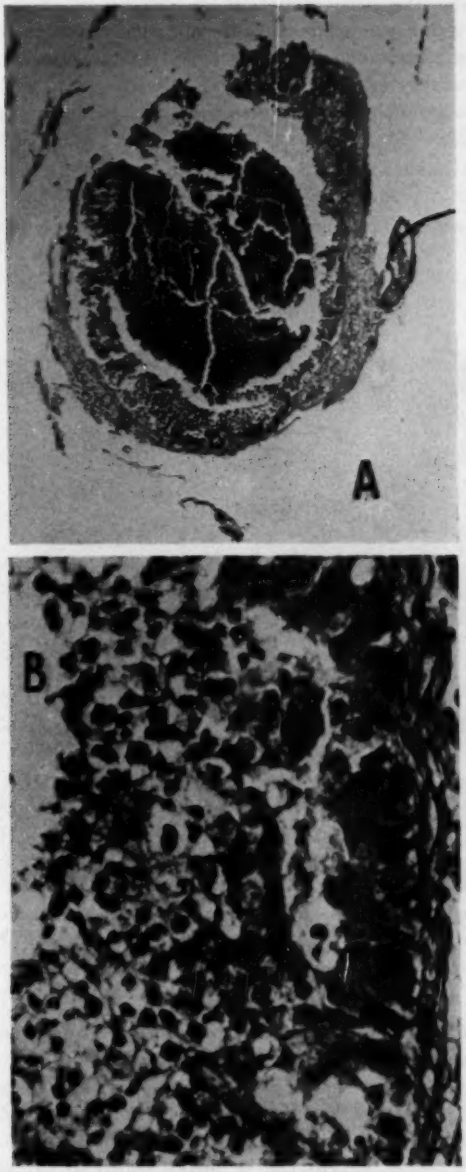

Fig. 5.—Nidation stage: *A*, bovine carcinoma of the kidney seven days after it had been transplanted to the anterior chamber of a guinea pig's eye. The nodule, surrounded by a thin stromal capsule from the host tissue, shows a necrotic core; the marginal layer (*B*) shows well preserved tumor cells. × 30. *B*, marginal portion of transplant shown in *A*. × 450.

plantation; inflammatory cell infiltration may be heavy. Figure 6*A* shows the nidation stage of a transplanted human adrenal-cortex carcinoma; the section, taken 20 days after the transplantation, shows a nest of tumor cells surrounded by a variety of inflammatory cells.

After nidation is established, the tissue changes in the transplant may follow one of three courses (Fig. 1):

1. If the host tissue offers strong resistance to growth, the transplant may be rapidly destroyed by a severe inflammatory reaction.

2. If the growth momentum of the tumor tissue is vigorous and the host resistance is low, the transplant proceeds directly to the stage of growth.

3. If both the growth momentum of the tissue and the resistance of the host are high, there is established a period of equilibrium, termed nidation arrest, which

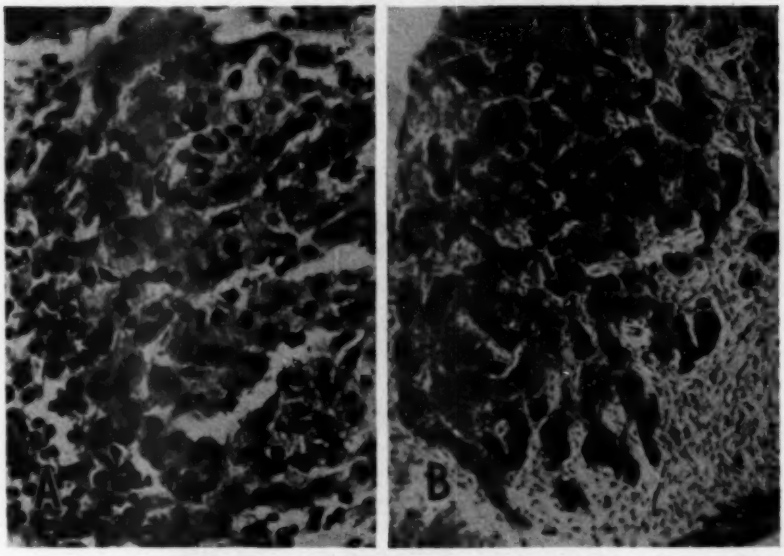

Fig. 6.—Nidation stage: *A*, human adrenal cortex carcinoma 20 days after it had been transplanted to a guinea pig's eye. The tumor cells are surrounded by a variety of inflammatory cells. × 420. *B*, nidation arrest: Equine adrenal pheochromocytoma 88 days after it had been transplanted to the anterior chamber of a guinea pig's eye. The section shows a nest of organized tumor cells attached to the iris. × 170.

may persist for months. Eventually this balance is destroyed and the transplant either emerges into the stage of growth or degenerates.

During nidation arrest a chronic low-grade inflammatory reaction is present at the site of transplantation. Grossly, as observed through the cornea, the vascularized transplant shows little change in size during this period. Histologically, organized clusters of tumor cells can be demonstrated in harvested transplants; viability of these cells is indicated by the fact that parallel transplants of the same tissue, observed further, often show an abrupt termination of the stage of arrested nidation and proceed to the stage of growth.

Figure 6*B* shows transplanted tissue from an adrenal pheochromocytoma of a horse in the state of nidation arrest; the transplant, harvested at 88 days, shows a nest of organized tumor cells attached to the iris.

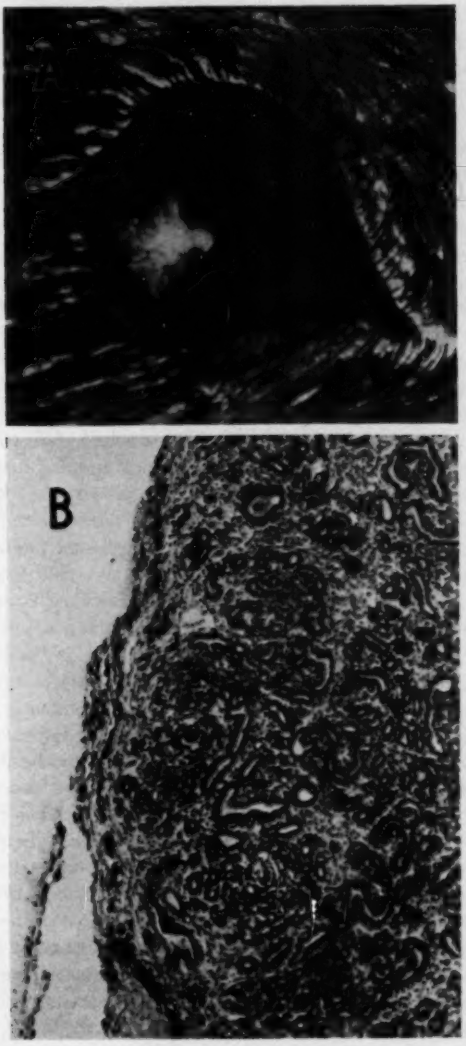

Fig. 7.—Growth stage: The gross appearance of a tumor transplant in the anterior chamber of the guinea-pig eye during the early period of growth; pseudopodial outgrowths appear at the margin of the vascularized transplant. × 6. *B*, carcinoma of the breast (C3H mouse) 10 days after it had been transplanted to the anterior chamber of a guinea pig's eye. The tumor tissue, growing outward from the iris, shows large, well differentiated glandular elements. × 70.

Growth Stage.—This stage is characterized by sustained growth of the organized tumor tissue in the transplant. Not only is there multiplication of tumor cells but there is proliferation of vessels and other stromal elements provided by the host. The rate of tumor growth, as observed through the cornea, is inversely proportional to the species difference between donor and recipient animal; the closer the species, the more rapid is the rate of growth. Transplants of Mouse Sarcoma 37 filled the anterior chamber in nine days; with tissue from a carcinoma of a cow a period of 24 days elapsed between the time that nidation ended and the anterior chamber became filled; with a human fibrosarcoma the anterior chamber became filled after 101 days of growth.

Grossly, the beginning of the stage of growth is often sharply marked by the appearance of pink pseudopodial projections at the margin of the transplant (Fig. 7*A*). However, the *in situ* observation of size increase, even threefold, is not in itself a reliable criterion for defining tumor growth. Initial enlargement is often the result of the inflammatory process which occurs in nidation. With sustained growth, the anterior chamber becomes filled; in no instance did growth occur beyond the limits of the anterior chamber during the present study. Growth of tumor tissue may be followed at any time by regression.

As pointed out by Greene,[4] differentiation of the tumor-tissue pattern commonly occurs during growth in the guinea pig. Carcinoma of the breast of the C3H mouse usually presents a compact cellular pattern with few acini. This tumor when grown in the guinea pig shows dilated acini filled with pink-staining material (Fig. 7*B*).

COMMENT

The pattern of the changes which occur in transplanted tumor tissue provides criteria for evaluating the results of tumor-growth experiments; this is particularly important in studies in which the anterior chamber has been employed. The lack of a comprehensive system of interpreting results has been a serious hindrance in this field of investigation. Results which are classified as "takes" or "positive" are often ambiguous because of the latitude these terms have required.

The gross appearance of transplants in the anterior chamber is deceptive; interpretation of results must be substantiated by histologic studies. The evaluation of anterior-chamber experiments is complicated, in particular, by the changes which occur during nidation. Changes occurring in this period are frequently interpreted as evidence of a "take"; this leads to significant statistical errors. The inflammatory enlargement observed grossly during nidation is often mistakenly recorded as tumor growth. The state of nidation arrest is frequently misinterpreted; small persisting transplants showing clusters of tumor cells on histologic examination are incorrectly recorded as "takes."

The uniform progression of transplants, through the stages of suspension, nidation, and growth, provides a comprehensive basis for evaluating results in anterior-chamber experiments. The results may be classified according to the maximal stage of development attained on transplantation. Accordingly, results may be divided into three groups:

1. Transplants attaining the stage of growth.
2. Transplants attaining the stage of nidation.
3. Transplants regressing during the stage of suspension.

This system of analysis has been applied in the study of 1,600 anterior-chamber transplantations which have been carried out in this laboratory to date.[2]

It is likely that when tumor tissue is transplanted to other sites changes occur which may be compared to those observed in the anterior chamber. A tumor fragment transferred to the subcutaneous tissue, the peritoneum, or the lumen of a vessel would at first be bathed in a fluid medium; this is comparable to the stage of suspension in the aqueous humor. Likewise, vascularization of the transplant and growth may occur in a manner similar to nidation and growth in the anterior chamber.

From this point of view the changes that a tumor transplant undergoes may reflect the biologic mechanisms involved in tumor embolism and metastasis. The tumor embolus, lodged in a new site, lives first in a tissue culture stage in the serum—the stage of suspension. If it survives, it becomes organized and vascularized—the stage of nidation. With proliferation of the organized tumor embolus the metastasis is established—the stage of growth.

The stage of nidation is probably the most critical phase in the evolvement of any transplant or embolus. During this phase the intrinsic growth momentum of the tumor cells is pitted against the resistance offered to growth by the tissue of the host at the site of nidation. We have shown that this contest may be prolonged for months—the end result being the ushering in of the stage of growth or the gradual absorption of the transplant.

This state of nidation arrest, as observed in our studies, deserves particular attention as it may help to explain one of the real mysteries observed clinically in the study of neoplasms—the delayed metastasis. It has been difficult to accept the idea that cancer cells can lie dormant for a period of years and then, as if coming out of hibernation, manifest their presence by forming a metastasis.

In the present experiments observations have brought forth tangible evidence that the survival of such a nest of tumor cells lodged in a foreign site does occur.

SUMMARY

Tumor tissue undergoes a consistent series of changes after transplantation. Analysis of this process is facilitated by employing the anterior-chamber technique and by using transplants of heterologous tumor tissue.

Observations indicate that three fundamental phases are involved in the establishment of a transplant. In the suspension stage temporary sustenance is provided for the transplanted tissue. In the nidation stage the tumor cells are integrated with the vascular system of the host. In the growth stage expansive proliferation of the organized tumor tissue occurs.

The pattern of tissue changes thus defined provides the basis for a clearer interpretation of transplantation experiments. Observation of this process may offer a key to the understanding of biologic mechanisms involved in tumor embolism and metastasis.

The illustrations were prepared by Joe D. Humphrey, Department of Pathology, Ohio State University College of Medicine.

EFFECT OF AUREOMYCIN ON TRANSPLANTED TUMORS

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IT HAS been reported that unpurified aureomycin exerts a growth-promoting activity when added in small amounts to the rations of hogs, chickens, and turkeys.[1] More recently, the effect of aureomycin on the growth of chicks was tested by supplementing the basal diet with 100 mg. and 50 mg. of crystalline aureomycin hydrochloride per kilogram of diet. It was observed that aureomycin exercises a growth-promoting effect both in the presence and in the absence of added vitamin B_{12} and that it had a "sparing effect" on vitamin-B_{12} requirement in some instances but not in others.[2] In view of the growth-stimulating activity of aureomycin and its extensive medical use it became advisable to test the effect of this antibiotic on the growth of cancers. The present report covers our investigations of the effect of aureomycin on transplanted tumors only. The effect on spontaneous tumors is still under investigation, and the results will be reported in due time.

August Rat Carcinoma 175-G, Crocker Rat Carcinoma, Mouse Sarcoma 180 and Mouse Adenocarcinoma Eo771 were used for this experimentation. In the first series of experiments the animals were given the dose of aureomycin which corresponded to the therapeutic dose, calculated by weight, prescribed in medical practice. Thus in one series of trials 1.6 mg. of aureomycin hydrochloride per 100 gm. of body weight was given daily for 4, 7, and 10 days. The total doses were, respectively, 6.4 mg., 11.2 mg., and 16 mg./100 gm. wt. These figures correspond to total therapeutic doses of 4.5 gm., 8 gm., and 11.2 gm., amounts which are often given to patients in various infectious diseases. In another series of trials the dose was doubled: 3.2 mg./100 gm. wt./day, also for 4, 7, and 10 days. These total doses correspond to 9, 16, and 22.4 gm., the total doses which are used in radiotherapeutic practice for the prevention of roentgen sickness.

From the Southern Bio-Research Laboratory, Florida Southern College.

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Presented at the Annual Meeting of the American Association for Cancer Research, Cleveland, April 28, 1951, and at the Annual Meeting of the American Society for Experimental Pathology, Cleveland, May 2, 1951.

1. Stokstad, E. L.; Jukes, T. H.; Pierce, J.; Page, A. C., Jr., and Franklin, A. L.: J. Biol. Chem. **180**:647, 1949.

2. Stokstad, E. L., and Jukes, T. H.: Proc. Soc. Exper. Biol. & Med. **76**:73, 1951.

In this range of total dose of aureomycin hydrochloride—from 6.4 mg. to 32 mg./100 gm. wt.—the growth-stimulating activity of the antibiotic was clearly noticeable as far as these four transplanted tumors were concerned.

The observations are summarized in the table.

Analysis of the figures of the table shows that although there was some variation in the rate of tumor acceleration with various doses of aureomycin hydrochloride, the difference is not significant. When we compare the figures for the same type of tumor, Rat Carcinoma 175-G, the total dose of 6.4 mg./100 gm. wt. gave an increase of 40% in size of tumor, while a total dose of 32.0 mg./100 gm. wt. gave an increase of 54% (Chart 1). In the case of Crocker Rat Carcinoma the acceleration of growth is more pronounced (Chart 2). On the other hand, in

Observations on Effect of Aureomycin on Transplanted Cancers

Type of Cancer	Animals	Total Dose of Aureomycin Hydrochloride, Mg./100 Gm. Wt.	Av. Size of Tumor After 3 Weeks, Cc.	Av. Size of Tumor After 4 Weeks, Cc.
Rat Carcinoma 175-G.....	20	6.4	9.1	18.9
Rat Carcinoma 175-G.....	20	Control	5.5	13.3
Rat Carcinoma 175-G.....	20	11.2	7.3	17.8
Rat Carcinoma 175-G.....	20	Control	3.9	12.3
Rat Carcinoma 175-G.....	15	12.8	10.3	20.9
Rat Carcinoma 175-G.....	15	Control	6.2	11.3
Rat Carcinoma 175-G.....	20	16.0	9.6	19.9
Rat Carcinoma 175-G.....	20	Control	6.5	13.4
Rat Carcinoma 175-G.....	15	22.4	12.2	21.5
Rat Carcinoma 175-G.....	15	Control	6.3	13.6
Rat Carcinoma 175-G.....	15	32.0	11.8	23.7
Rat Carcinoma 175-G.....	15	Control	6.9	14.8
Crocker Rat Carcinoma.....	20	11.2	15.6	29.8
Crocker Rat Carcinoma.....	20	Control	9.3	14.5
Crocker Rat Carcinoma.....	20	32.0	13.9	28.4
Crocker Rat Carcinoma.....	20	Control	8.8	15.2
Mouse Sarcoma 180.....	20	11.2	4.2	6.3
Mouse Sarcoma 180.....	20	Control	3.1	4.2
Mouse Sarcoma 180.....	20	32.0	4.5	6.8
Mouse Sarcoma 180.....	20	Control	3.3	4.1
Mouse Carcinoma Eo771....	20	11.2	5.5	12.8
Mouse Carcinoma Eo771....	20	Control	4.1	9.0
Mouse Carcinoma Eo771....	20	32.0	6.0	14.2
Mouse Carcinoma Eo771....	20	Control	4.8	11.2

the case of Mouse Sarcoma 180 the total dose 11.2 mg./100 gm. wt. induced an increase of 50%, and the total dose of 32 mg./100 gm. wt., an increase of 61% in size of tumor. All in all, we may say that when animal bearers of the aforementioned transplanted tumors were treated with total doses of aureomycin hydrochloride which correspond to the total doses used in medical practice (from 4.5 gm. to 22 gm.) the results seemed to indicate that aureomycin definitely enhances tumor growth.

In our next series of experiments we administered much larger doses of aureomycin hydrochloride: a total dose of 144 mg./100 gm. wt., or four and one-half times the maximum total therapeutic dose, corresponding to about 100 gm. of aureomycin hydrochloride given to humans.

Thirty rats were inoculated with Crocker Carcinoma and divided into two groups of 15 animals each. The next day, when the transplant was 1 day old, the first group of rats was given 8 mg./100 gm. wt. daily for 18 consecutive days. At the end of three weeks the average size of the tumors was only 3.7 cc., compared with 9.9 cc. for the controls. However, these

figures include seven rats in which there was complete disappearance of the transplant. During the fourth and fifth weeks after transplantation the remaining tumors in this group regained the tempo of their growth, and at the end of the fifth week the average size of eight tumors was 44.2 cc., compared with 32.3 cc. for the controls.

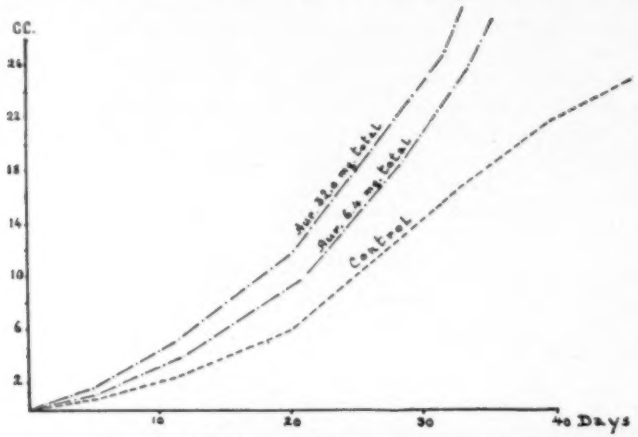

Chart 1.—Effect of aureomycin hydrochloride on the growth of Rat Carcinoma 175-G. The curves represent the increase in size of tumor transplants in 40 treated rats and 40 controls. The average weight of the rats was 155 gm. The total doses of aureomycin hydrochloride were 6.4 and 32 mg. per 100 gm. of body weight.

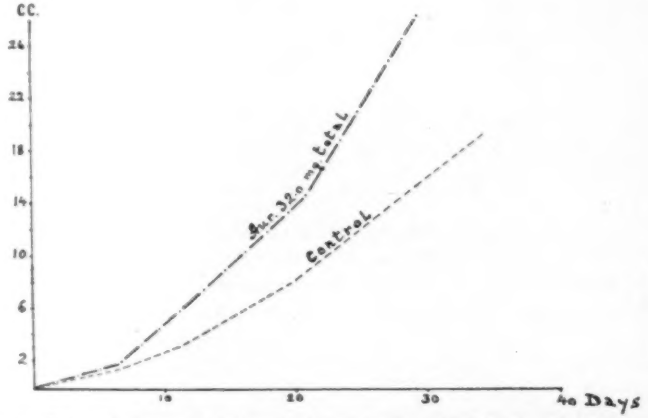

Chart 2.—Effect of aureomycin hydrochloride on the growth of Crocker Rat Carcinoma. The curves show the increase of growth in tumor transplants in 20 treated rats and 20 controls. The average weight of the rats was 148 gm. The dose of aureomycin hydrochloride was 32 mg./100 gm. wt.

In the next series of experiments 40 rats were divided into two groups and inoculated with the same tumor. Administration of aureomycin hydrochloride began when the transplant was 3 days old. The total dose was the same: 144 mg./100 gm. wt. In this group of treated animals there was observed complete disappearance of five transplants. The average size of tumors at

the end of three weeks was 4.3 cc., compared with 8.3 cc. for the controls. In this case the remaining 15 tumors also regained their growth at the end of the fifth week following transplantation. The average size for 14 tumors (one rat died) was 46.6 cc., compared with 33.1 cc. for 18 controls (two rats died).

In the next series of experiments 30 bearers of 10-day-old tumors (Crocker Carcinoma) were divided into two groups. At that time the average size of a tumor was 3.3 cc. for the first group and 3.6 cc. for the control group. The rats of the first group were given the same daily dose of aureomycin hydrochloride, 8 mg./100 gm. wt., for 18 days. No regression was observed in this group. At the end of the treatment, four weeks after transplantation, the average size of the tumors of the treated group was 20.5 cc., compared with 17.7 cc. for the controls (Chart 3). Thus it appears that the administration of heavy doses of aureomycin does not produce inhibition of the tumor which is well advanced in its growth, at least as far as Crocker Rat Carcinoma is concerned. On the other hand, the inhibitory effect of heavy doses of aureomycin on the transplant 1 to 3 days old is not unlike the effect which guanazolo exerts on similar transplants.[3]

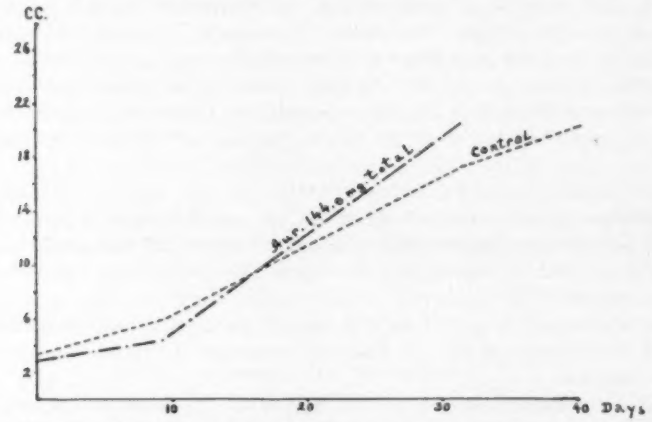

Chart 3.—Effect of aureomycin hydrochloride on well-advanced growth of Crocker Rat Carcinoma. The curves show the increase of growth in 10-day-old tumor transplants in 15 treated rats and 15 controls. The average weight of the rats was 164 gm. The total dose of aureomycin hydrochloride was 144 mg./100 gm. wt.

While the rats tolerated quite well the "therapeutic dose" not exceeding 32.0 mg. (total), the heavy total dose of 144 mg./100 gm. wt. produced a definite toxic effect. There was a loss of weight (average, 15.5%), with pronounced sluggishness and apathy. It is worth while noting that the span of life of the rats receiving the "therapeutic dose" of aureomycin hydrochloride was slightly higher than that of the controls in spite of the increase of growth (5.3%), while the rats receiving massive doses of the antibiotic succumbed earlier than the controls (average, 20.5%). This fact seemingly indicates that heavy doses of aureomycin do produce a toxic effect on rats and mice and that the temporary inhibition of growth might have been induced by the ill-effect of this preparation.

3. Sokoloff, B.; Redd, J. B., and Dutcher, R.: Metabolite Antagonists in Experimental Cancer: Guanazolo, Arch. Path. **50**:411 (Oct.) 1950.

The investigation concerning the effect of aureomycin on spontaneous tumors is not yet completed. But there is some accumulated evidence that aureomycin given in therapeutic doses enhances the growth of spontaneous mammary cancer in C3H mice.

COMMENT

The mechanism of the growth-promoting activity of aureomycin is not yet defined. Whether the antibiotic has a "sparing" effect on vitamin B_{12}, and thus stimulates growth indirectly, or whether it exerts this activity through some other physiological channel, the fact remains that when administered in "therapeutic doses" it enhances the growth of some transplanted tumors. We believe that this fact deserves the full attention of the medical profession and further investigation along these lines. This is of practical importance owing to the fact that since Howland and associates [4] reported the protective action of aureomycin against radiation, this antibiotic is being used in radiotherapeutic practice for the prevention of roentgen sickness. In a number of instances it is prescribed to patients affected with cancer or in postoperative use of radiation. It is advisable, we feel, that on the basis of the data here reported, more caution should be exercised in administering aureomycin to patients affected with cancer or suspected to have cancer till more is known about the mechanism of its growth-promoting activity.

SUMMARY

Aureomycin given in daily doses of 1.6 mg. and 3.2 mg./100 gm. body wt. for 4, 7, and 10 days, respectively, enhanced the growth of transplanted tumors, Rat Carcinoma 175-G, Crocker Rat Carcinoma, Mouse Sarcoma 180 and Mouse Adenocarcinoma Eo771.

Aureomycin given in daily doses of 8 mg./100 gm. body wt. for 18 days induced complete disappearance of the 1 to 3-day-old transplant of Crocker Rat Carcinoma in some instances.

Aureomycin given in daily doses of 8 mg./100 gm. body wt. for 18 days to the rat bearers of 10-day-old transplants of Crocker Rat Carcinoma had no inhibitory effect on tumor growth. A slight enhancing effect was observed in tumor growth in this experiment.

NOTE.—Our paper was in press when the work of W. F. Loomis, "On the Mechanism of Action of Aureomycin" (*Science* **111**:474, 1950) was brought to our attention. The author suggests that the toxicity of aureomycin is derived from its ability to inhibit aerobic phosphorylation. This observation might explain the retarding effect of massive doses of aureomycin on transplanted tumors.

4. Howland, J. W.; Furth, F.; Bennett, L. R.; Coulter, M., and McDonnel, G. M.: Effect of Aureomycin and Antibiotics on Whole Body Irradiation, Atomic Energy Commission, AECU 748, (July) 1950.

FLAVONOIDS IN EXPERIMENTAL CANCERS

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FIRST Griffith and associates,[1] and after them Field and Rekers,[2] and Sokoloff, Redd and Dutcher,[3] demonstrated experimentally that certain flavonoid compounds give protection against radiation injury. The mortality of rats and dogs submitted to a total-body, near-lethal dose of radiation was greatly reduced, and hemorrhages typical of the radiation illness syndrome were absent or almost absent. Subsequently, the clinical investigation by Arons, Freeman, and Oppenheim,[4] and Miller, Hurt, and others [5] revealed the fact that certain flavonoid compounds prevent to a considerable degree the appearance of radiation erythema, having, however, only a moderate effect on nausea and vomiting. From the clinical evidence so far available there is no indication that flavonoid therapy interferes with the radiation effect on cancer growth. The fact that the flavonoids are now being used in radiotherapeutic practice, mostly in cancer patients, necessitated an investigation of the effect of flavonoid compounds on experimental cancer.

EXPERIMENTAL PROCEDURE

Three types of transplanted tumors were selected for this experimentation: Rat Carcinoma 175-G, Crocker Rat Carcinoma, and Mouse Sarcoma 180. A flavonoid compound similar to the original vitamin P of Szent-Györgyi was given to rats and mice daily by subcutaneous injection in doses of 2.0, 5.0, and 10 mg./100 gm. body wt. for 20 days, or a total dose of 40, 100, and 200 mg., respectively. The total dose of 40 mg./100 gm. wt. corresponds to the total dose of flavonoid compound, estimated by weight, given to cancer patients during the course of radiotherapy (600 mg. daily for 30 to 40 days). No ill effect was observed in normal rats from any of these doses; there was no loss in weight or any other manifestation indicative of toxicity of the compound.

From the Southern Bio-Research Laboratory, Florida Southern College.

This work was aided by a grant from the Marcia Tucker Foundation and the U. S. Vitamin Company.

Presented at the Annual Meeting of the American Association for Cancer Research, Cleveland, April 28, 1951, and the Annual Meeting of the American Society for Experimental Pathology, Cleveland, May 2, 1951.

1. Griffith, J. Q., Jr.; Anthony, E.; Pendergrass, E. P., and Perryman, R.: Proc. Soc. Exper. Biol. & Med. **64**:332, 1947.

2. Field, J. B., and Rekers, P. E.: J. Clin. Invest. **28**:746, 1949.

3. Sokoloff, B.; Redd, J. B., and Dutcher, R.: Science **112**:112, 1950; Proc. Soc. Exper. Biol. & Med. **75**:6, 1950; J. Clin. Invest. **30**:395, 1951.

4. Arons, I.; Freeman, J., and Oppenheim, A.: Cancer Research **11**:238, 1951.

5. Miller, T. R.; Hurt, F. R., and others: J. Clin. Invest. **30**:399, 1951.

EFFECT OF FLAVONOIDS ON TRANSPLANTED TUMORS

In all experiments conducted with these three types of tumors the administration of the flavonoid compound resulted in a moderate inhibition of the growth as illustrated in Charts 1, 2, and 3.

Chart 1 represents the results of experimentation on 100 animals, 40 of which served as controls. The total dose of 40 mg./100 gm. wt. produced a retardation of growth of 32%, the total dose of 100 mg./100 gm. wt. a retardation of 39%, and the total dose of 200 mg./100 gm. wt. a retardation of 59% of the average size of tumor reached in the controls.

Even more definite results were obtained with Crocker Rat Carcinoma (Chart 3). In this case the average size of the tumors of the animals given 40 mg./100 gm. wt. was 49% smaller than that of the control tumors. The total dose of 100 mg./100 gm. wt. produced a still stronger retardation, 59%, and the total dose of 200

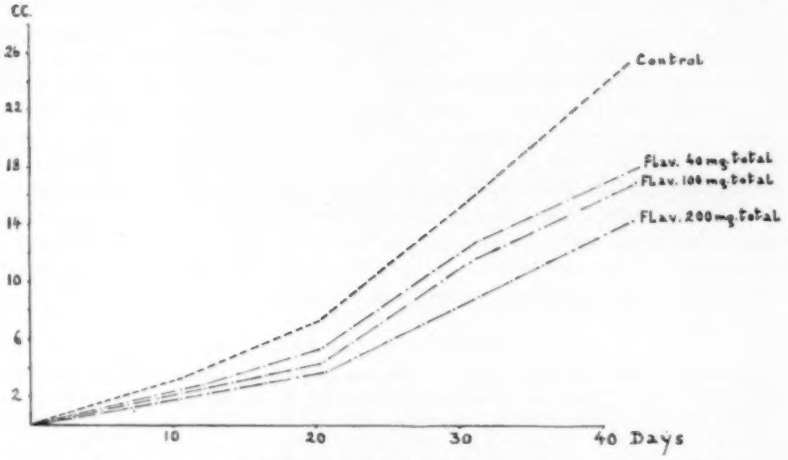

Chart 1.—The effect of "therapeutic" doses of flavonoids on Rat Carcinoma 175-G.

mg./100 gm. wt. resulted in an 80% retardation of growth. This experiment covers 100 animals, 40 of which were used as controls.

Chart 3 refers to the experimentation on Mouse Sarcoma 180. The results obtained may be summarized as follows: The total doses of 40, 100, and 200 mg./100 gm. wt. produced retardations of growth of 26, 38, and 51%, respectively, as compared with the control figures. This experiment covers 75 animals, 30 of which served as controls.

The analysis of these results suggests that a larger dose of the flavonoid compound tends to produce a stronger retarding effect on the above-mentioned transplanted tumors. In all three series of experiments the total dose of 200 mg./100 gm. wt. was the most effective as far as retardation of growth was concerned. This dose of the flavonoid compound is nontoxic, according to our own tests.

The fact which deserves some attention is that after the flavonoid therapy was discontinued the tumors of the treated animals continued to grow more slowly than

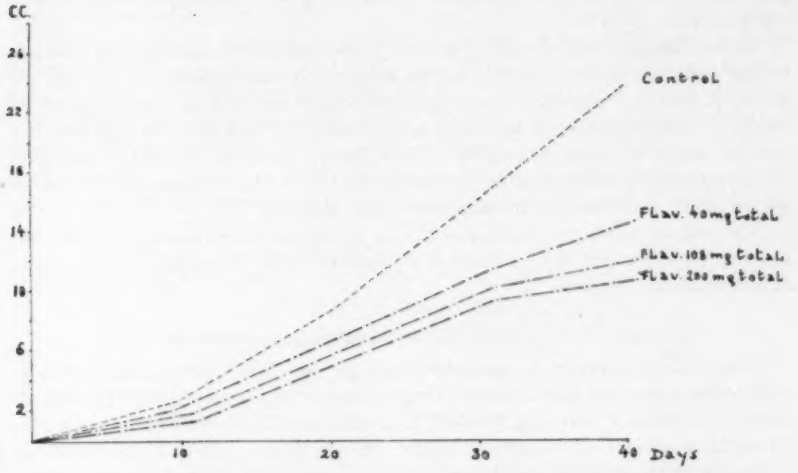

Chart 2.—The effect of "therapeutic" doses of flavonoids on Crocker Rat Carcinoma.

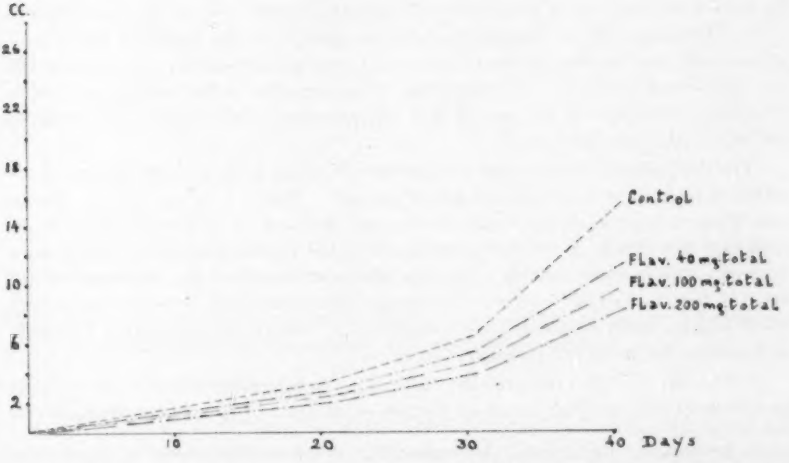

Chart 3.—The effect of "therapeutic" doses of flavonoids on Mouse Sarcoma 180.

those of the controls. Two weeks afterward the average tumor size for the treated animals was still below that for the controls. Such was not the case with guanazolo. Immediately after the administration of that drug was discontinued, the tumors regained their growth.[6]

Thus it appears that the administration of the flavonoid compound has a certain lasting effect on tumor growth. In this respect it is worth while noting that the flavonoid therapy resulted in some prolongation of the span of life of cancerous animals. The extension of the life span amounted to 26% for rats and 19% for mice as compared with the controls. These figures, however, should be taken with some reservation, owing to the fact that at the end of experimentation the tumors were smaller in the treated animals than in the controls.

In order to find a clue to the mechanism of the inhibitory activity of flavonoids and the possibility that they are related to adrenal cortex hormones, a new series of experiments was initiated.

EFFECT OF FLAVONOIDS ON ADRENALECTOMIZED RATS

There are apparently no published data on the distribution of flavonoids in mammalian tissue and no reference to the presence of these compounds in the adrenal gland. Two of us (Eddy and Sokoloff [7]) were able to discover the presence of two flavonoid compounds in the adrenal gland. One compound present in the amount of 2 mg. per gram of dry tissue showed a typical curve with the borocitrate reaction, with the maximum at 450 mμ. The other flavonoid was disclosed in a smaller amount, about 1 mg. per gram of dry tissue, with the maximum at 400 mμ. Both of these compounds gave a positive coloring with the Lorenz reaction. The flavonoids are present not only in the adrenal gland of the cow but in that of man as well. We were able to examine the adrenal glands of two babies, 7 and 9 mo., respectively, and to disclose the presence of a similar compound (maximum at 400 mμ borocitrate reaction). Furthermore, it appears that a flavonoid compound is present in the spleen in an amount of 2 mg. per gram of dry tissue (maximum at 400 mμ borocitrate reaction).[7a]

The disclosure of adrenal and splenic flavonoids led us to an investigation of the effect of flavonoids on adrenalectomized animals. It is a well known fact that in rats bilateral adrenalectomy results in survival of about 15 to 30% of the animals. This high percentage of survival is explained by the relative abundance of accessory adrenal tissue in these animals. For this reason we observed the adrenalectomized rats for two weeks prior to flavonoid therapy and selected for the experiment only those which clearly manifested the symptoms of cortical insufficiency. The table summarizes the results of this investigation.

Altogether 22 rats were used for this test. The adrenalectomized rats receiving no flavonoid therapy died within an average of 28 days. The adrenalectomized rats

6. Sokoloff, B.; Redd, J. B., and Dutcher, R.: Metabolite Antagonists in Experimental Cancer: Guanazolo, Arch. Path. **50**:411 (Oct.) 1950.

7. Eddy, W. H., and Sokoloff, B.: Report to the Annual Meeting of the American Institute of Nutrition, May 3, 1951, Cleveland.

7a. The borocitrate and Lorenz reactions have been described by B. T. Sokoloff and J. B. Redd (Study on Vitamin 'P': II. The Chemical Nature and Physiological Activity of Vitamin P Factors, Monograph 1, Florida Southern College, Lakeland, Fla., Florida Southern College Press, 1949.

given 5 mg./100 gm. body wt. daily (after the two weeks of observation had elapsed) survived 56 days (an average for 12 rats), a small but definite extension of the life span. Females gave a somewhat higher rate of survival than males in both groups of animals, treated and control.

This experiment was repeated on the same number of rats (12 given flavonoid therapy and 10 serving as controls). The dose of the flavonoid compound was the same as in the previous trial: 5 mg./100 gm. body wt. daily with an average body weight of 159 gm. In this series the average life span for treated rats was 52 days, while the average life span for controls was 31 days.

From these experiments it appears that the flavonoid compound prolongs the life span of adrenalectomized rats to some degree. Its action seems to be similar to

Effect of Flavonoids on the Longevity of Adrenalectomized Rats

Rat *	Daily Dose of Flavonoid Compound, Mg./100 Gm. Body Wt.	Duration of Life After Operation, Days †
1, f.	5	62
3, m.	5	50
4, m.	5	45
6, f.	5	68
7, f.	5	59
8, f.	5	57
9, m.	5	55
10, m.	5	53
11, f.	5	58
12, f.	5	61
13, m.	5	51
14, m.	5	54
Control		
18, f.	0	35
20, f.	0	34
25, m.	0	21
26, m.	0	31
29, f.	0	22
30, f.	0	22
32, f.	0	28
33, m.	0	30
34, m.	0	26
39, f.	0	31

* A British breed of rats was used; 12 were treated, and 10 served as controls. Their average weight was 151 gm. The sex distribution was 50-50.

† The average longevity of the treated rats was 56 days; that of the controls, 28 days.

that of adrenal cortex extract and cortisone but to a much less pronounced degree as far as the expansion of the life span of adrenalectomized animals is concerned.[8]

COMMENT

In his report entitled "The Causal and Formal Genesis of Cancer," presented to the Cancer Conference in London in 1928, the late James Ewing pointed out that little if any attention is paid to the possible role which intercellular substance might play in the genesis of cancer. Now, 23 years after this statement, the situation remains unchanged. The attention and efforts of investigators in the cancer field have been directed almost exclusively to the biochemistry of cells and nuclei of

8. Selye, H., and Schenken, V.: Proc. Soc. Exper. Biol. & Med. **39**:518, 1938.

cancerous tissue. Numerous papers have appeared and still are appearing on the modifications of enzymic and metabolic changes in the nuclei of cells submitted to carcinogenic treatment, but the question of the intercellular substance and its role, if any, in the cancerization of tissue is left untouched. And yet, as Leo Loeb [9] remarked, the forces promoting and restraining growth of the organism might be closely related to the activity of this cell-binding substance. Once the binding properties of intercellular substance are changed, the cellular organization as such is disrupted, and, as in the case of cancerous growth, almost nonexistent. Some experimental data point to a possible role of intercellular substance in carcinogenesis as a phenomenon precursory to cancerization of cells.

McCutcheon, Coman, and Moore [10] found that the mutual adhesiveness of human carcinoma cells is less than that of corresponding normal cells. According to Irving Zeidman,[11] the administration of methylcholanthrene, a carcinogenic substance, decreases the adhesiveness of epithelial cells. It produces some chemical changes in intercellular substance. These changes seem to be associated with a decrease in calcium, for the adhesiveness of cells can be reduced by calcium-free diffusion (Zeidman).

On the other hand, Hyman and Chambers [12] disclosed that adrenal-cortex hormones directly affect intercellular substance by "thickening" it and thus apparently increasing the adhesiveness of the cells. Puig-Musset [13] in his turn found that flavonoids increase the binding properties of intercellular substance by "thickening" it and augmenting the adhesiveness of cells. These observations, as limited as they might be, indicate that adrenal-cortex hormones might play some role in the regulation of the binding properties of intercellular substance perhaps in conjunction with the coenzymic activity of flavonoids. It is in the light of this theory that the inhibitory effect of flavonoids on tumor growth might find its interpretation.

SUMMARY

The flavonoid compound given to rat bearers of Carcinoma 175-G and Crocker Rat Carcinoma and to mice bearers of Sarcoma 180 in a total dose of 40, 100, and 200 mg./100 gm. body wt., respectively, produced a moderate inhibitory effect on tumor growth.

The flavonoid compound administered in these doses to normal animals has no toxic effect.

The flavonoid compound given to adrenalectomized rats in a daily dose of 5 mg./100 gm. wt. prolonged their life span.

9. Loeb, L.: The Biological Basis of Individuality, Springfield, Ill., Charles C Thomas, Publisher, 1945.

10. McCutcheon, M.; Coman, D. R., and Moore, F. B.: Cancer **1**:460, 1948.

11. Zeidman, I.: Cancer Research **7**:386, 1947.

12. Hyman, C., and Chambers, R.: Endocrinology **32**:310, 1943.

13. Puig-Musset, P.: Introduction al estudio de la vitamin P Barcelona, Migel Servet Company, 1945.

CALCIFIED LESIONS IN C3H MICE GIVEN PURIFIED LOW-PROTEIN DIETS

Tissues Involved: Heart, Skeletal Muscles, Arteries, and Lungs

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AND
FLOYD S. DAFT, Ph.D.
BETHESDA, MD.

A STUDY was made of the effects of a diet low in protein and deficient in choline on the incidence of hepatomas induced by carbon tetrachloride in C3H mice.[1] This report is concerned with extensive necrosis and calcification of cardiac and skeletal muscles, arterial and pulmonary calcification, and other pathologic changes occurring in C3H mice given such a diet. Supplementing the diet with 2 to 10 mg. of choline chloride per cubic centimeter of drinking water or offering 3 mg. of alpha-tocopherol weekly by supplement cup did not prevent the lesions to be described.

MATERIALS AND METHODS

The approximate percentage composition of the deficient diet (No. 1926) was as follows: sucrose, 86.05; casein, 4; Osborne and Mendel salt mixture, 4; Wesson oil, 3; cod-liver oil, 2; cystine, 0.5; anhydrous dextrose mixture, 0.25 (0.25 gm. of this mixture contained 1 mg. of riboflavin); p-aminobenzoic acid, 0.1; and inositol, 0.1. To each 100 gm. of the above diet were added 3 mg. of calcium pantothenate, 1 mg. of nicotinic acid, and 0.5 mg. each of thiamine hydrochloride, and pyridoxine hydrochloride.

In the initial experiments a series of C3H mice was restricted to the deficient diet immediately after being weaned at 22 to 29 days of age; nearly all died in from four to 10 weeks. In subsequent experiments three series of weanling mice were fed a commercial dog chow stock diet[2] for 72, 79, and 134 days, respectively, before receiving the deficient diet, No. 1926. After two weeks on Diet 1926, the mice in the third series and over one-half of those in the second series received 0.5% and 2% carbon tetrachloride in olive oil (0.1 cc. per 20 gm. of mouse) twice weekly by stomach tube. Survivors were killed for study serially at three to five months after beginning the deficient diet.

Autopsies were performed as soon as feasible on most of the animals that died and on all killed for study. The tissues from 236 mice receiving the deficient diet were saved for histologic study and were fixed in a 4.5% solution of formaldehyde. Paraffin sections of the organs were stained routinely with azure eosinate and frequently for calcium and hemosiderin. Van Gieson's solution of trinitrophenol and acid fuchsin and other stains were used when indicated. Frozen sections of the hearts, livers, kidneys, or adrenals of 128 mice were stained for fat with oil red O.[2a]

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Federal Security Agency.

1. Highman, B., and Eschenbrenner, A. B.: Influence of Choline on the Incidence of Carbon Tetrachloride Hepatomas in C3H Mice, presented at the Forty-Second Annual Meeting of the American Association for Cancer Research, Inc., Cleveland, Ohio, April 28, 1951.

2. The dog chow used was that produced by the Ralston Purina Company, St. Louis.

2a. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.

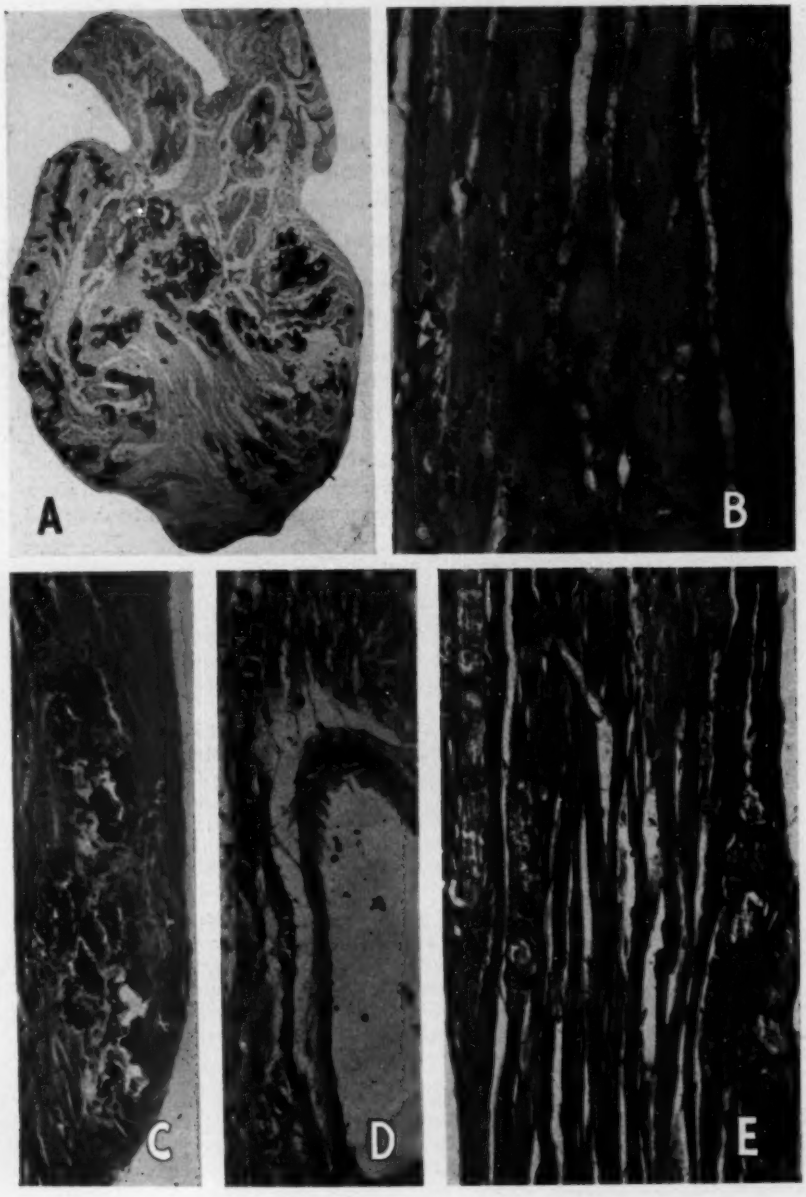

Figure 1

(See legends on opposite page)

RESULTS

The mice receiving the deficient diet failed to gain or lost weight; their fur became shaggy and lusterless, and in many a persistent mild to severe diarrhea developed. The rate and the average length of survival were less in males than in females, but there were no significant sex differences in the histopathologic findings. The severity of the pathologic changes often varied considerably in individual mice receiving the diet for similar periods, but the cardiac lesions tended to be more severe in the mice given the deficient diet at weaning (Fig. 1*A*). Carbon tetrachloride did not alter significantly the lesions to be described in organs other than the liver.

PATHOLOGIC OBSERVATIONS

Heart.—In some mice, particularly those dying within two weeks after commencing the deficient diet, there were often only a few scattered single and small groups of necrotic myocardial fibers evoking no apparent inflammatory reaction (Fig. 1*B*). These fibers showed a few to many basophilic granules giving the Prussian blue reaction for iron and staining like calcium with hematoxylin, alizarin red S, and von Kossa's method. There were also occasional fibers variably showing some fine fat droplets, a slight decrease in cytoplasmic oxyphilia, indistinct cell borders and cross striations, and pale vesicular nuclei sometimes containing a central rodlike chromatin mass as seen in Anitschkow myocytes. Similar lesions were seen occasionally in control mice given a stock diet with carbon tetrachloride.

The hearts of nearly all the mice that died or were killed after 30 days presented at autopsy pale-yellow or white ventricular patches and streaks. Microscopically, there were few to many necrotic foci scattered throughout the ventricular myocardium and occasionally extending into the epicardium or the endocardium (Fig. 1*C*). The atria were seldom involved, and the mitral and aortic valves rarely showed small marginal calcified areas. The foci were frequently elongated in the axis of the bordering muscle fibers; the largest lesions exceeded 1 mm. in length. The necrotic muscle fibers were usually basophilic and hyalinized, but sometimes granular in appearance, and often fragmented. Nearly all the necrotic fibers stained like calcium, and some, particularly the granular forms and those along the periphery of the lesions, also stained like hemosiderin. In older lesions (animals survived from two to five months) there was often considerable organization with mono-

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EXPLANATION OF FIG. 1

Fig. 1.—*A*, section of the heart of a mouse given the deficient diet, No. 1926, supplemented with choline at weaning. The mouse was dead in 53 days. Note the extensive calcification in the ventricles. Von Kossa's stain; × 15.

B, heart of a mouse fed the stock diet for 72 days followed by the deficient diet, No. 1926, for four days. Note early lesions with two partially calcified muscle fibers on the right and a stippled fiber in the left lower portion of the section. Azure eosinate; × 400.

C, heart of a mouse fed the stock diet for 134 days followed by Diet 1926 supplemented with choline for 153 days. Note the calcified ventricular lesion near the epicardial surface. Azure eosinate; × 200.

D, heart of a mouse fed Diet 1926 for 72 days after being weaned. Note dark calcified myocardial fibers and a calcified plaque in the media of a large branch of the coronary artery. Azure eosinate; × 200.

E, skeletal muscles adjoining vertebrae. Note the necrosis of striated muscle fibers with relatively slight cellular reaction. The sections have been decalcified, and the affected fibers appear gray instead of black as in the heart. Same mouse as in *D*. Azure eosinate; × 200.

nuclear cells, macrophages, fibroblasts, and some collagen or noncollagenous fibrillar matrix between the necrotic muscle fibers and along the periphery of the lesions. There was no cellular reaction in the surrounding tissue. Except for the frequent occurrence of slight venous distention and engorgement, there were no significant vascular changes in the necrotic areas. The hearts of a few mice showed patchy areas of fatty degeneration, and a few showed small areas of calcification in an occasional artery as described below (Fig. 1*D*).

Muscles.—The lesions of the skeletal muscles when severe were characterized grossly by silvery streaks, which were usually most marked in the recti capitis and posterior femoral muscles. Microscopically, there were few to many lesions involving isolated segments and small or large groups of muscle fibers. The changes were essentially similar to those described in the myocardial foci, but relatively fewer muscle fibers were affected usually, and the cellular reaction between the necrotic and calcified muscle fibers tended to be greater. The necrotic fibers were often swollen, contorted, and fragmented, and the segments were frequently marginated by mononuclear cells grading into multinucleated syncytial masses and giant cells (Fig. 1*E*). Extensive necrosis and calcification were found occasionally in the striated musculature of the esophagus (Fig. 2*A*). Less extensive similar lesions of the skeletal muscles were seen occasionally in control mice fed a stock diet and receiving carbon tetrachloride.

Arteries.—Arterial lesions were found commonly in the kidney, the pancreas, and the retroperitoneal fatty tissue, and occasionally in the lungs, the heart, the liver, the spleen and other organs; lesions were infrequent in mice receiving the diet for less than 60 days. The larger arteries at the hilus and the corticomedullary junction of the kidney were most frequently affected (Fig. 2*B*), but in advanced cases many of the smaller arteries were also involved. Some vessels showed no apparent involvement or only isolated intimal lesions, while others in the same section showed multiple lesions in one or more segments, some involving the entire thickness of the wall. The most frequent early alteration was the localized deposition in the intima of some basophilic homogeneous material, staining like calcium and sometimes also like hemosiderin, producing a slight to prominent irregular elevation or plaque, which was usually covered by endothelium. In some instances, short segments of elastic fibers in the underlying media were coated with this basophilic material. In other instances the affected portion of the media had a relatively homogeneous refractile basophilic appearance, while elastic fibers were indistinct and muscle fibers were absent. The lesions were usually sharply delineated, but a few basophilic granules staining like calcium were seen occasionally in some of the neighboring cells. There were generally no changes in the adventitia and surrounding tissues. In some instances, particularly in older lesions, the basophilic material often showed some variation in depth of color and was sometimes distinctly granular or refractile in appearance. In routine sections (not decalcified) it was often broken into variably sized plates with serrated borders. The rigid calcareous character of some the lesions was evidenced further by absence of the usual intimal wrinkles, due to postmortem contraction, on the inner surface overlying the lesions. The lesions were generally confined to arteries, but in some instances, because of the extent of involvement, identification of the vessel as an artery was uncertain.

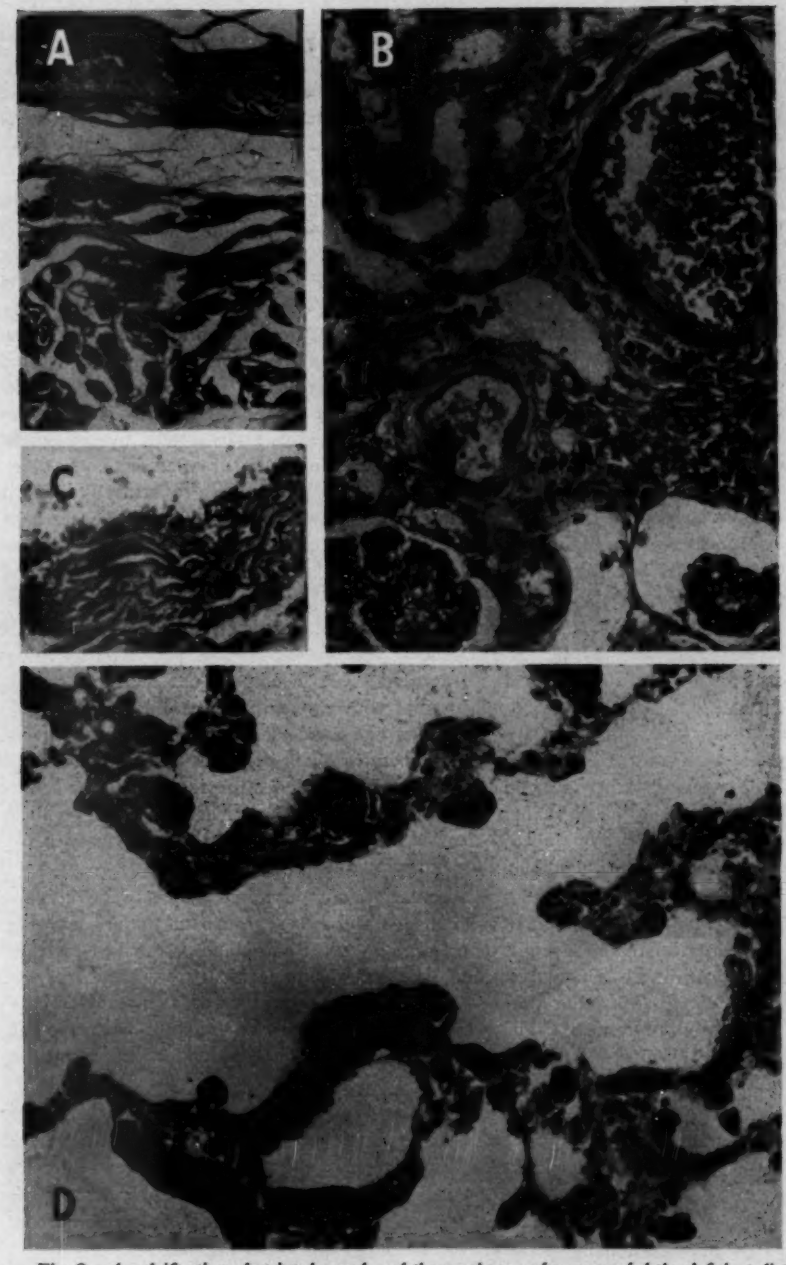

Fig. 2.—*A*, calcification of striated muscles of the esophagus of a mouse fed the deficient diet, No. 1926, for 135 days. Calcified fibers appear dark. Azure eosinate; × 300.

B, calcification of arteries in a kidney of a mouse fed the deficient diet for 153 days. Azure eosinate; × 300.

C, section of the aorta of a mouse fed the deficient diet for 98 days. Note focal calcification involving elastic fibers in the media. Azure eosinate; × 300.

D, lungs of a mouse fed the deficient diet for 198 days. Note extensive calcification in alveolar ducts and alveoli. Azure eosinate; × 300.

In the lungs, lesions were seen occasionally in the pulmonary veins, involving the myocardium-like fibers of the media. In the aorta and the pulmonary artery, focal lesions were seen occasionally in the intima and media. The elastic fibers were coated, displaced, or disorganized by the basophilic calcified material (Fig. 2*C*).

Lungs.—The lungs of mice receiving the deficient diet for more than 60 days often showed extensive deposition of a homogeneous basophilic material that stained like calcium and occasionally like hemosiderin. The deposits were particularly heavy along the surface of the alveolar ducts and in alveoli adjoining ducts (Fig. 2*D*). They occurred chiefly as irregular plaques covered, at least in many instances, by the cytoplasm of lining septal cells; the latter often contained a few basophilic granules. The interalveolar septa were frequently thickened, and some contained round or irregular basophilic calcareous bodies, from 5 to more than 50 microns in diameter, showing frequently a pale or vacuolated center. The alveolar spaces occasionally contained a few calcareous masses and necrotic, hyalinized, or deeply basophilic alveolar phagocytes, some clinging to the alveolar septa. In older animals, the deposits tended to be less homogeneous and were frequently broken up into variably sized plates with serrated margins. No calcified deposits were seen in bronchi or bronchioles, except that the matrix of the bronchial cartilages sometimes stained like calcium. Only a few of the larger arteries and veins showed calcified lesions as described previously.

Liver.—In the mice receiving the deficient diet hepatic cirrhosis developed, with abundant fat and ceroid. The cirrhosis was similar to that produced by other choline-deficient diets [3] and was prevented (only in animals not receiving carbon tetrachloride) by supplementing the diet with choline. The ceroid deposits were frequently admixed with material staining like hemosiderin or calcium. Many livers also had scattered single and conglomerate small rounded basophilic calcified bodies, some evidently derived from necrotic ceroid-laden macrophages. The bodies were sometimes encapsulated or marginated by mononuclear cells and cellular fibrous tissue. In a few mice there was focal calcification of the liver capsule. No hepatomas were found in the mice receiving the deficient diet.

Kidney.—In addition to the vascular lesions described above, some kidneys had few to many scattered single tubules and small groups of tubules showing calcification of the epithelium, or occasionally some basophilic or calcified material in the lumen. A few had calcium deposits within or along the surface of the pelvis (Fig. 3*A*). Some kidneys showed focal scarring and areas of tubular fatty degeneration or necrosis. The perirenal fat was largely of the embryonic type and occasionally showed small areas of necrosis, calcification, and mononuclear-cell infiltration (Fig. 3*B*).

Other Changes.—Many mice, particularly those that died, had obvious depletion of lipoid in the adrenal cortex, severe pneumonia, multiple abscesses in various organs, or other incidental infections and lesions. Slight to moderate hemosiderosis was seen in the spleen and occasionally in the liver and in the epithelium of the duodenal villi. Ceroid was found frequently in the zona reticularis of the adrenal cortex and occasionally in the spleen, the lungs and other organs.

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3. Lillie, R. D.; Ashburn, L. L.; Sebrell, W. H.; Daft, F. S., and Lowry, J. V.: Histogenesis and Repair of the Hepatic Cirrhosis in Rats Produced on Low Protein Diets and Preventable with Choline, Pub. Health Rep. **57**:502-508, 1942.

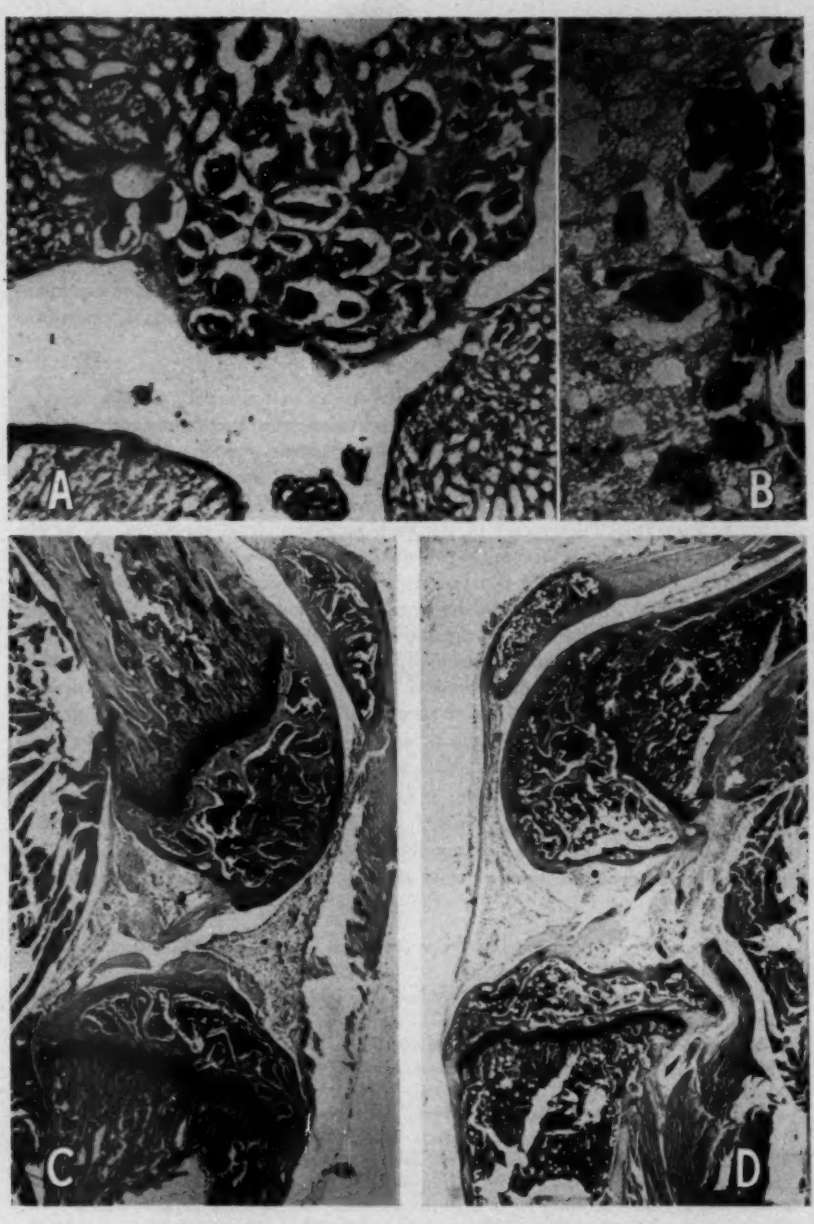

Fig. 3.—*A*, calcification involving pelvis and tubules in the medulla of a kidney of a mouse fed the deficient diet, No. 1926, for 153 days. Azure eosinate; × 300.

B, calcification in perirenal fat in mouse fed the deficient diet for 153 days. Azure eosinate; × 300.

C, knee of a mouse fed the stock diet for 36 days after weaning. Azure eosinate; × 20.

D, knee of a mouse fed the deficient diet for 36 days after weaning. Note particularly narrowing of the epiphysial-cartilage plates and reduction in the number and the size of the trabeculae in the metaphyses. Azure eosinate; × 20.

The thyroid and parathyroid glands and the long bones (after decalcification) of a relatively small number of mice fed the deficient diet were studied. The thyroid follicles were generally lined by flattened or cuboidal epithelium and contained abundant colloid. No hypertrophy was noted either grossly or microscopically in the parathyroids. The long bones usually revealed evidence of impaired growth (Figs. 3*C* and *D*). The epiphysial cartilage plate was frequently narrowed and deeply basophilic, suggesting calcification. The metaphysis showed relatively few or no trabeculae. The thickness of the cortex was reduced. The marrow usually appeared normal in cellularity. The peripheral blood was studied in a few animals. No striking changes were noted in the white cells. The erythrocyte count was usually normal, but sometimes slightly or moderately reduced.

COMMENT

The necrotic and calcareous lesions reported here are not due apparently to a deficiency in choline, since they were not prevented by supplements of choline. While the skeletal lesions in our mice did not respond apparently to supplements of alpha-tocopherol (vitamin E),[4] it is possible that the latter was poorly absorbed, owing to functional impairment of the gastrointestinal tract, as suggested by the frequent occurrence of diarrhea. We found less extensive similar lesions of the skeletal muscles occasionally in apparently healthy, well-nourished control C3H mice fed a stock diet and receiving carbon tetrachloride. Dunn, Morris, and Dubnik[5] found similar lesions of the skeletal muscles frequently in C3H mice fed a thiamine-deficient diet and occasionally in emaciated pair-fed control animals. These findings suggest that the lesions of the muscles may be induced occasionally in C3H mice by many types of stress causing prolonged impairment of health.

The lesions described here differ in part, particularly in the variety of tissues affected, from somewhat similar lesions produced by certain other abnormal diets.[6] For example, the lesions in the arteries and muscles resembled those observed by Ashburn, Daft, Endicott, and Sebrell[6a] in rats given sulfaguanidine in purified diets. In the rats, however, no comparable lesions were found in the heart or the lungs, the coronary arteries were chiefly affected, and the muscle lesions responded to vitamin E.[6b]

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4. Pappenheimer, A. M.: Muscular Dystrophy in Mice on Vitamin E-Deficient Diet, Am. J. Path. **18**:169-181, 1942.

5. Dunn, T. B.; Morris, H. P., and Dubnik, C. S.: Lesions of Chronic Thiamine Deficiency in Mice, J. Nat. Cancer Inst. **8**:139-155, 1947.

6. (*a*) Ashburn, L. L.; Daft, F. S.; Endicott, K. M., and Sebrell, W. H.: Lesions in Rats Given Sulfaguanidine in Purified Diets, Pub. Health Rep. **57**:1883-1891, 1942. (*b*) Daft, F. S.; Endicott, K. M.; Ashburn, L. L., and Sebrell, W. H.: Vitamin E Deficiency in Rats Given Succinyl Sulfathiazole in Purified Diets, Proc. Soc. Exper. Biol. & Med. **53**:130-131, 1943. (*c*) Heston, W. E.; Larsen, C. D., and Deringer, M. K.: Variations in Occurrence of Pathologic Calcification, Nephritis, and Amyloidosis in Mice Fed Control and Modified Diets, J. Nat. Cancer Inst. **6**:41-47, 1945. (*d*) White, J., and Mider, G. B.: The Effect of Dietary Cystine on the Reaction of Dilute Brown Mice to Methylcholanthrene (Preliminary Report), ibid. **2**:95-97, 1942. (*e*) Butler, M.: Experimental Calcification in Mice, Proc. New York Path. Soc. **24**:79-86, 1924. (*f*) Bragdon, J. H., and Levine, H. D.: Myocarditis in Vitamin E-Deficient Rabbits, Am. J. Path. **25**:265-272, 1949. (*g*) Harris, P. N., and Wulzen, R. M.: Pathologic Changes Induced in Guinea-Pigs by Administration of Diets Deficient in the Anti-Stiffness Factor, ibid. **26**:595-616, 1950.

The lesions in the lungs of our mice are somewhat similar to those found by Heston, Larsen, and Deringer [6c] in Strain A mice fed a low cystine-low protein diet. Feeding a similar low-cystine diet containing methylcholanthrene to dilute-brown mice, White and Mider [6d] found extensive calcification involving particularly the media of the aorta, but they did not report any significant changes in the heart and skeletal muscles. The diet used in our experiments was not deficient in cystine.

In many respects the lesions in our experimental mice resembled metastatic calcification such as that found in hypervitaminosis D.[7] Although Diet 1926 has no apparent excess vitamin D, it is possible that the metabolism of compounds like the vitamin D sterols may be disturbed in animals fed abnormal diets.

SUMMARY

Weanling C3H mice fed a low-protein diet deficient in choline usually died within 10 weeks. Mice fed a stock diet for two to five months before beginning the deficient diet were more resistant. In addition to the anticipated cirrhosis, fatty changes, and ceroid deposition in the liver, the mice fed the deficient diet revealed widespread lesions somewhat resembling metastatic calcification such as occurs in hypervitaminosis D. Microscopically, there were scattered focal lesions throughout the ventricular myocardium, rarely in the auricles, showing hyalinization, necrosis, and calcification of myocardial fibers with little or no inflammatory reaction and no associated vascular lesions. Similar lesions occurred in the skeletal muscles, particularly the recti capitis. In the lungs, calcareous deposits were found in the bronchial cartilages and interalveolar septa and along the surface of alveolar ducts and alveoli. Calcareous deposits were also found in the intima and media of many arteries, particularly in the kidney, and occasionally in the renal pelvis, retroperitoneal fat, esophagus, and other tissues. Necrosis and calcification were not prevented by supplementing the diet with choline or alpha-tocopherol (vitamin E).

7. Shohl, A. T.; Goldblatt, H., and Brown, H. B.: The Pathological Effects upon Rats of Excess Irradiated Ergosterol, J. Clin. Invest. **8**:505-531, 1930. Harris, L. J., and Innes, J. R. M.: The Mode of Action of Vitamin D; Studies in Hypervitaminosis D; The Influence of the Calcium-Phosphate Intake, Biochem. J. **25**:367-390, 1931.

SELECTIVE PANCREATIC ACINAR DESTRUCTION BY dl-ETHIONINE

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THE DISCOVERY of toxic substances that are cell specific in their action has provided new tools for research. Examples of such substances are the goitrogenic agents, thiouracil and thiourea, which inhibit thyroxine formation in the thyroid,[1] and alloxan, which selectively destroys the beta cells of the pancreatic islets.[2] The present report deals with another such compound, ethionine, the administration of which, interestingly enough, leads to destruction of pancreatic acinous tissue without structural alteration of the encompassed islands of Langerhans. The genesis of the pancreatic lesion induced by ethionine and its effects on other organs are described here.

EXPERIMENTAL PROCEDURES

One hundred and twenty-five female rats of the Curtis-Dunning strain, weighing approximately 200 gm., were maintained on a commercial chow[3] supplemented with lettuce. *dl*-ethionine was injected by the intraperitoneal route. Each injection consisted of 50 mg. of *dl*-ethionine dissolved in 2 cc. of saline solution. Groups of four to 10 rats were killed 4, 8, 12, and 24 hr. after a single injection. Those killed at 48 and 72 hr. received two and three injections, respectively. The rats killed at intervals of 5, 7, 14, 21, and 25 days received, daily, a single injection of 50 mg. of *dl*-ethionine. The rats were anesthetized with pentobarbital sodium (1 cc. of a 0.2 per cent solution) and exsanguinated. Representative portions of numerous organs (liver, kidney, salivary glands, lungs, spleen, lymph nodes, heart and aorta, esophagus, stomach, small and large intestine, pancreas, adrenals, thyroids, ovaries, and uterine tubes) were removed and fixed in 4% neutral formaldehyde solution. The tissues were embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin-eosin and with Masson's trichrome stain. Frozen sections of the kidney, the liver, the pancreas, and the adrenals were stained with Lillie's hematoxylin-oil red O.

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A preliminary report of some of the findings has appeared elsewhere (Goldberg, R. C.; Chaikoff, I. L., and Dodge, A.: Destruction of Pancreatic Acinar Tissue by DL-Ethionine, Proc. Soc. Exper. Biol. & Med. **74**:838, 1950).

1. Franklin, A. L.; Chaikoff, I. L., and Lerner, S. R.: The Influence of Goitrogenic Substances on the Conversion in Vitro of Inorganic Iodide to Thyroxine and Diiodotyrosine by Thyroid Tissue with Radioactive Iodine as Indicator, J. Biol. Chem. **153**:151, 1944.

2. Dunn, S. J.; Sheehan, H. L., and McLetchie, N. G. B.: Necrosis of Islets of Langerhans Produced Experimentally, Lancet **244**:484, 1943.

3. The chow used was purina® laboratory chow produced by the Ralston Purina Company, St. Louis.

The total fatty-acid contents of selected livers were determined by a method described elsewhere.[4] Blood and urine glucose measurements were made by the procedure of Hassid.[5]

RESULTS

First Day.—The earliest cytological changes were observed in liver and kidney. An increase in histologically demonstrable lipid appeared in the parenchymal cells of the liver as early as four to eight hours after a single injection of 50 mg. of

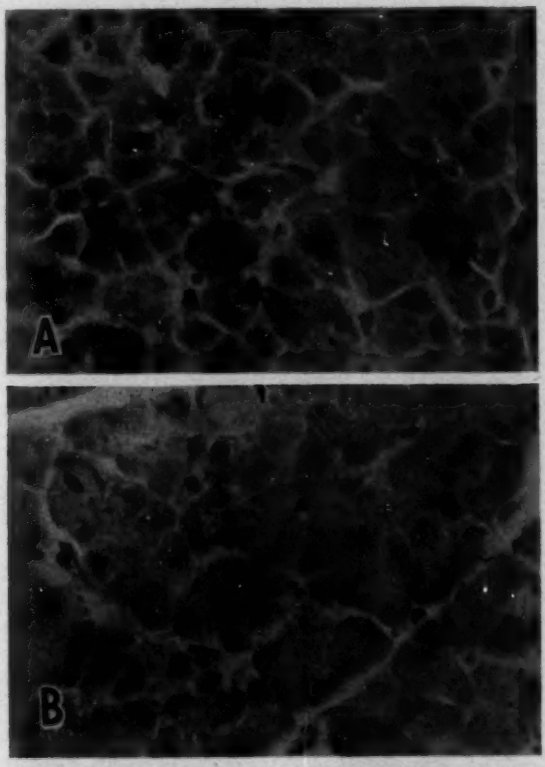

Fig. 1.—*A*, normal rat pancreas. The acini are well filled with zymogen granules, and the infranuclear region of the cells is intensely basophilic. Masson; × 500.

B, pancreas from a rat killed 12 hr. after injection of 50 mg. of *dl*-ethionine. There is obvious reduction in cytoplasmic basophilia and degranulation of the acinous tissue. Masson; × 500.

ethionine. At that time an increased amount of stainable fat was found in the proximal convoluted tubules of the kidney.

4. Chaikoff, I. L., and Kaplan, A.: The Blood Lipids in Completely Depancreatized Dogs Maintained with Insulin, J. Biol. Chem. **106**:267, 1934.

5. Hassid, W. Z.: Determination of Reducing Sugars and Sucrose in Plants, Indust. & Engin. Chem. **8**:138, 1936. Somogyi, M.: A Method for the Preparation of Blood Filtrate for the Determination of Sugar, J. Biol. Chem. **86**:655, 1930.

By the time 12 hr. had elapsed, the acinar cells of the pancreas showed a significant decrease in the characteristic basophilia located at the base of the cells (Fig. 1*A* and *B*). The zymogen content was also reduced in four animals of this group, but areas of well-granulated acini were found in the glands of the other six rats of this group.

At 12 hr., vastly increased amounts of lipid were found in the liver, the cells containing many small fat globules (Fig. 2*A* and *B*). Nine and 11 per cent total

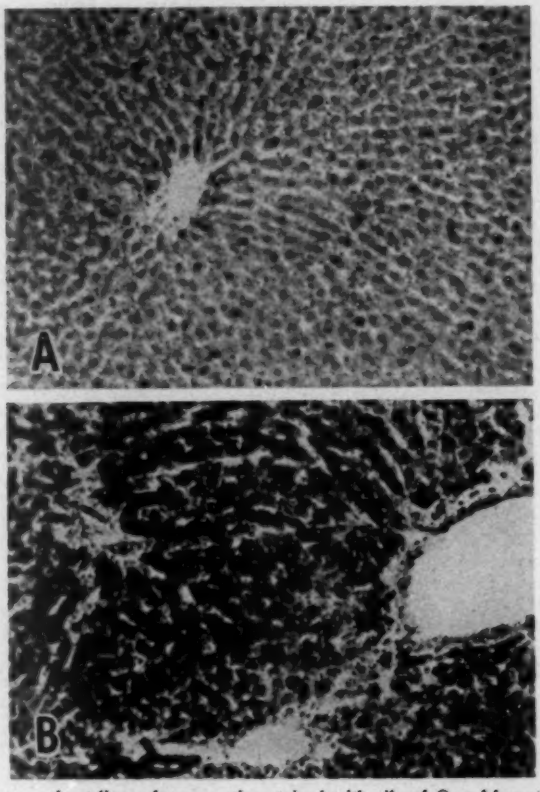

Fig. 2.—*A*, normal rat liver; frozen section stained with oil red O and hematoxylin; × 100. Only an occasional cell reveals lipid droplets.

B, liver from a rat killed 12 hr. after a single injection of 50 mg. of *dl*-ethionine. All the parenchymal cells are filled with small lipid globules. Oil red O and hematoxylin; × 100.

fatty acids were found in the livers of two of the treated rats; the normal values ranged from 2 to 5 per cent.

In 12 hr. the amount of stainable lipid in the basilar part of the cells of the proximal convoluted tubules of the kidney was also increased. An obvious decrease in the lipid content of the zona fasciculata of the adrenal cortex, with no significant change in the zona glomerulosa, was also observed at this interval (Fig. 3*A* and *B*). The former zone was hyperemic.

Twenty-Four to Thirty-Six Hours.—The livers of all eight rats studied at this interval revealed diffuse massive fatty change. Values as high as 18 to 20% total fatty acids were found by chemical analysis. The liver nuclei were enlarged, and many contained one to four extremely large acidophilic nucleoli. In all but two of the animals no indication of nuclear degeneration was observed; in these, karyolysis, pyknosis, and some fragmentation were seen in occasional cells.

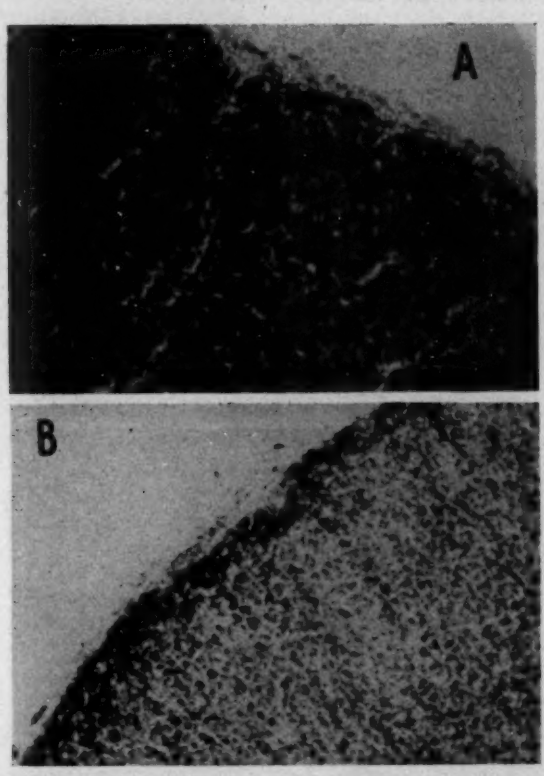

Fig. 3.—*A*, normal rat adrenal; oil red O and hematoxylin; × 100. *B*, adrenal from a rat killed 12 hr. after a single injection of *dl*-ethionine. Note the loss of lipid from the zona fasciculata with little or no change in the zona glomerulosa. Oil red O and hematoxylin; × 100.

No pathological alterations were observed in the kidney other than a fatty change in the proximal convoluted tubules described above. The adrenals, however, were definitely hyperemic, but the lipid content was now normal. Hemorrhagic adrenals were found in four of the eight animals.

Variable amounts of zymogen were present in the pancreatic acinar cells. In four animals the glands were completely devoid of zymogen. In the glands that still contained zymogen granules, the acini immediately surrounding the islets of Langerhans were well granulated, lending a "halo" appearance to the islets. In

some areas the acinar cells were undergoing vacuolar degeneration. Only a small percentage of these vacuoles gave a positive reaction for lipid. Edema and hyperemia were evident. Necrosis, leucocytic infiltration, and fibrin deposition were seen in some areas along with an almost complete loss of normal acinar architecture.

Considerable hemosiderosis was observed in the spleen, and the mesenteric lymph nodes contained large amounts of yellow pigment.

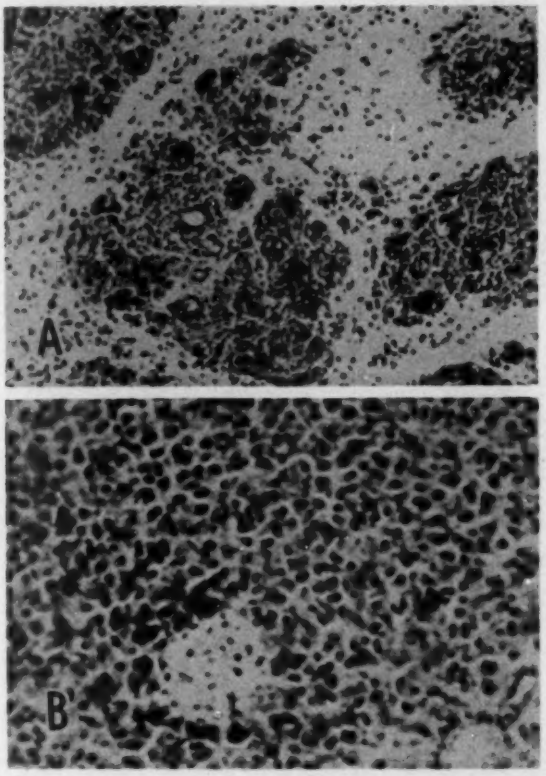

Fig. 4.—*A*, pancreas from rat treated for three days with *dl*-ethionine. Edema, leucocytic infiltration, glandular degeneration, atrophy, and fibroblastic activity are evident. Hematoxylin and eosin; × 100.

B, pancreas from another rat, killed after three days of *dl*-ethionine treatment, revealing only loss of acinar architecture, nuclear pyknosis, and uneven zymogen degranulation. Masson; × 150.

Forty-Eight to Seventy-Two Hours.—The changes described above were now more severe. The liver cells were filled with lipid globules, and in many cells they had coalesced to form one or two large globules. The hepatic nuclei were quite enlarged and still exhibited immense acidophilic nucleoli.

The adrenals were still hyperemic, and the amount of lipid in the pars fasciculata appeared to be slightly decreased. Hemorrhagic adrenals were frequent.

The pancreas now presented a picture of advanced degeneration. It was edematous and infiltrated with leucocytes. Polymorphonuclear cells were present in great numbers. The high percentage of eosinophils was quite striking. The interstitial tissue was also congested with plasma cells, macrophages, and fibroblasts; fibrin deposition was prominent, and new fibrous tissue was being laid down. Hydropic degeneration and necrosis of the acinous tissue were evident in many areas (Fig. 4*A*), and their nuclei showed pyknosis, karyolysis, and karyorrhexis. The pancreases from three of the eight animals of this group did not show marked

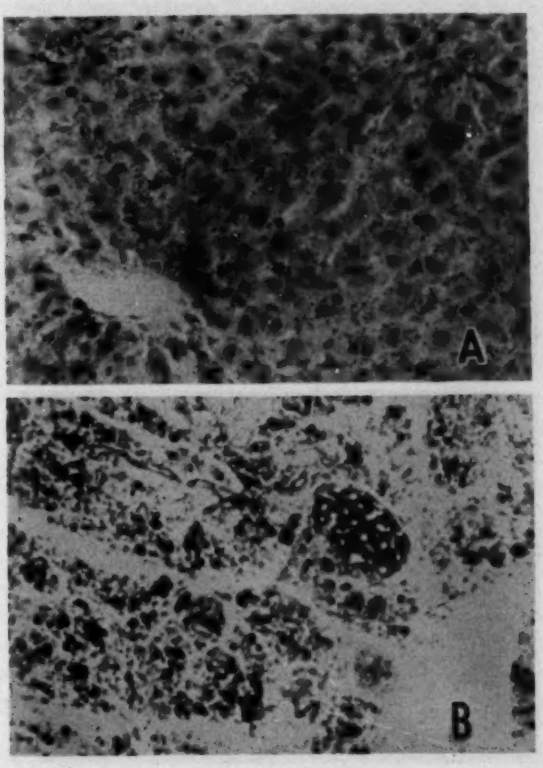

Fig. 5.—*A*, liver from rat killed seven days after inauguration of *dl*-ethionine treatment The liver parenchymal cells are now almost completely devoid of stainable fat, while the Kupffer cells are choked with lipid. Oil red O and hematoxylin; × 200.

B, edematous pancreas with only scattered cells containing zymogen-like material (intense black in photograph) from a rat killed after two weeks of *dl*-ethionine treatment. An islet can be seen to the right of center. Masson; × 100.

degenerative changes and were composed of morphologically unaltered acini, which in many areas still contained considerable amounts of zymogen-like material, particularly those acini surrounding islets (Fig. 4*B*).

Five Days to One Week.—Liver lipids had begun to decline, so that at the end of this interval only small amounts of histologically demonstrable fat were revealed

in the hepatic parenchyma of most of the animals. The Kupffer cells, on the other hand, were laden with small lipid globules (Fig. 5*A*). Chemical determinations on two livers revealed a fatty-acid content of 3 and 5%, respectively. No signs of liver degeneration or necrosis were observed. The adrenal lipid content was within the limits of normal variation, but the hemorrhagic and hyperemic conditions observed at earlier intervals were not dissipated. Microscopic examination of the pancreas revealed a picture similar to that seen at three days. Very little zymogen could be

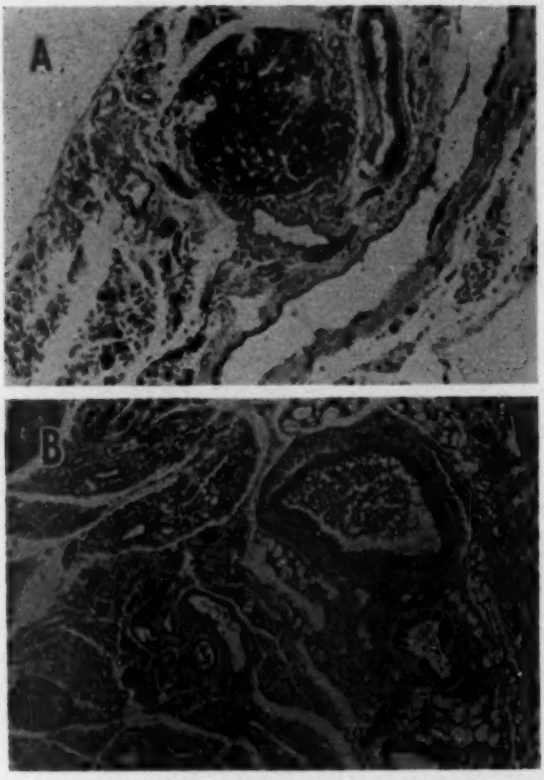

Fig. 6.—*A*, intact islet of Langerhans surrounded by dense fibrous tissue which has replaced the exocrine pancreas in a rat killed after three weeks of *dl*-ethionine treatment. A few intact cells containing zymogen-like material can be seen in the lower left. Masson; × 100.

B, pancreas from rat killed after three weeks of *dl*-ethionine treatment. Note fatty infiltration, dilated ducts, dense fibrous tissue, and intact islets in the upper and lower left corners. Masson; × 100.

found in any of the glands, although in some there remained many areas of intact cells.

Two Weeks.—The pancreas presented a picture of scattered acinar epithelial cells, both single and in groups of three to four cells (Fig. 5*B*). Some of these cells, embedded in the newly formed collagenous tissue, contained a zymogen-like material

Capillary sprouts were proliferating in the newly organized tissue, and capillary and arteriolar dilatation was prominent. Many of the ducts were dilated, and some contained an inspissated eosinophilic material. The gland was shrunken and contracted, even though still quite edematous, and a subacute inflammatory reaction was noted in three of the eight animals. The pancreas of two rats still contained some areas of relatively intact acinous tissue. The liver presented a picture similar to that observed at the one-week interval, i. e., a very small amount of fat in the hepatic parenchymal cells, and Kupffer cells filled with small lipid globules.

Three Weeks.—What was formerly the pancreas was now seen to be an area of dense fibrous tissue in which were embedded the intact islets of Langerhans, ducts, fibroblasts, leucocytes, plasma cells, macrophages, and cells of an undetermined nature resembling acinar cells, some of which contained acidophilic granular material (Fig. 6*A* and *B*). The kidney still contained fat in the proximal convoluted tubules, but no degenerative changes other than this were observed in the kidney.

No pathological alterations were observed at any interval in the islets of Langerhans, the respiratory tract, the cardiovascular system, the mucosa of the gastrointestinal tract, the salivary glands, the thyroid and parathyroids, the ovaries, or the uterine tubes. Urine and blood glucose analyses failed to reveal any evidence of diabetes. When 50 mg. of *dl*-ethionine was injected intraperitoneally daily, a mortality of less than 6% was observed.

COMMENT

The first cytological change noted in the pancreatic acinous tissue was the loss of the characteristic infranuclear cytoplasmic basophilia. This basophilia is considered to reflect ribonucleoprotein content.[6] Fibrosis appeared after this zymogen degranulation and loss of basophilia. The occurrence of an acute inflammatory reaction and extensive necrosis was not consistently found, and therefore cannot be considered an integral aspect in the genesis of the pancreatic lesion. In contrast to the hepatic lesion,[7] no sex difference was observed in the destructive action of ethionine on acinous tissue.[8]

On the other hand, the rapidly developing fatty liver is characterized by the absence of necrosis or other marked degenerative changes. Interestingly enough, the fat content of the liver returns to normal in a week or more despite continued injections of ethionine. At this time the Kupffer cells were engorged with fat.

The only other pathological sequelae of ethionine administration are the presence of lipid in the proximal convoluted tubules of the kidney and hyperemia and loss of lipid in the adrenal cortex (zona fasciculata). The latter reaction may well be an adrenal cortical response to stress.

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6. Greenstein, J. P.: Nucleoproteins: Advances in Protein Chemistry, Vol. 1, New York, Academic Press, Inc., 1944, p. 207.

7. Farber, E.; Koch-Weser, D., and Popper, H.: The Influence of Sex and of Testosterone upon Fatty Liver Due to Ethionine, Endocrinology **48**:205, 1951. Jensen, D.; Chaikoff, I. L., and Tarver, H.: The Ethionine-Induced Fatty Liver: Dosage, Prevention, and Structural Specificity, J. Biol. Chem. to be published.

8. Goldberg, R. C.; Chaikoff, I. L., and Dodge, A. H.: Destruction of Pancreatic Acinar Tissue by DL-Ethionine, Proc. Soc. Exper. Biol. & Med. **74**:869, 1950. Farber, E., and Popper, H.: Production of Acute Pancreatitis with Ethionine and Its Prevention by Methionine, ibid. **74**:838, 1950.

The remarkable immunity of the islets of Langerhans emphasizes the specificity of ethionine action on acinous tissue. At no time was there any cytological evidence of islet damage or any indication of dysfunction as judged by urine and blood glucose determinations.

SUMMARY

The effect of the administration of *dl*-ethionine was studied in rats from as early as four hours after a single intraperitoneal injection of 50 mg. to as late as 25 days after chronic administrations of 50 mg. daily.

Pathological alterations were observed in the acinous portion of the pancreas, the liver, the kidney, and the adrenal glands.

Zymogen degranulation and a loss of basal basophilia in the acinous portion of the pancreas were observed as early as eight to 12 hr. after the first injection. In three to five days inflammation, edema, necrosis, and fibroblastic activity were evident. Marked fibrosis was evident when two to three weeks had elapsed. The pancreatic ducts were unaffected.

No cytological changes were observed in the islands of Langerhans at any interval. No evidence of diabetes was detected.

An increase in histologically demonstrable fat was observed in the liver as early as eight hours after a single injection. The liver became intensely fatty in 24 hr. after a single injection. Within one week the lipid content of the liver had returned to normal despite continued injections of ethionine.

THE HUMAN AORTA

IV. The Aorta in Diabetes Mellitus

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THE LATE manifestations of diabetes are gaining in importance with the increasing duration of the disease. Studies of patients whose diabetes was of more than 15-yr. duration [1] have shown that the most important risk at this stage of the disease is complications from the vascular system. Knowledge concerning the type and the extension of vascular sclerosis in the diabetic patient as compared with other individuals of like age and cardiovascular status is, however, scarce. The attention of studies of diabetic sclerosis has practically always been centered on the organs where the sclerosis gives rise to clinical manifestations: the retina, the kidneys, and the arteries of the extremities. The behavior of the aorta has received little attention. Bjørnsson,[2] in his thesis, in 1941, noted a slightly higher degree of sclerosis in the aortas of diabetic individuals than in the controls. This was to be expected when the higher level of serum cholesterol associated with diabetes was taken into consideration.

With the low mortality from diabetes in the early stage, especially among the younger age groups, only few diabetics go to autopsy before some degree of arterial hypertension has developed. This implies that valid results from comparing the degrees of sclerosis in a diabetic and a normal group can be obtained only when the control material contains a group of aortas from hypertensive patients without diabetes.

In a former paper,[3] the degree of sclerosis measured by the dry weight, the total cholesterol, and the total calcium was studied, and a suitable mode of treating the data was evolved. This material contained vessels from both normotensive and hypertensive patients and should therefore be suitable for comparison with diabetic

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From the Copenhagen County Hospital Medical Department F and the Finsen Laboratory.

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1. Hanssen, P.: Diabetes Mellitus in Bergen 1925-1942, Acta med. scandinav., 1946, Supp. 178. Poulsen, J. E.: Rep. Steno Memorial Hospital **4**:60, 1951. Root, H. F.: Diabetes and Vascular Disease in Youth, Am. J. M. Sc. **217**:545, 1949. Reuting, R. E.: Progress Notes on Fifty Diabetic Patients Followed Twenty-Five or More Years, Arch. Int. Med. **86**:891 (Dec.) 1950.

2. Bjørnsson, J.: Arteriosclerosis: A Chemical and Statistical Study, Diss., Copenhagen, 1941.

3. Faber, M., and Lund, F.: The Human Aorta: Influence of Obesity on the Development of Arteriosclerosis in the Human Aorta, Arch. Path. **48**:351 (Oct.) 1949.

vessels. In the former paper the data were grouped according to body weight. The results showed that no significant difference could be found between individuals of normal weight and the obese. Therefore these two groups are pooled in the normal and the hypertensive control material.

The material of diabetic patients consists of 32 aortas collected mostly at the same time as the normal control material, but discarded during the treatment of these groups. The age and the sex of the patients and the duration of the diabetes when known are shown in Tables 1 and 2. It will be seen that the diabetics do not represent simply a group of senile patients, the age distribution being practically the same as that found in the control material.

Among these 32 diabetic patients only four could be classified as having normal blood pressure. The rest had hypertension, either diagnosed by measuring blood pressure before death or recognized by hypertrophy of the heart at autopsy as calculated according to the data of Zeek.[4]

The treatment of the data follows the principles laid down in our former paper. The normal values of the logarithms of the three factors were calculated for each patient according to age by means of the formulas evolved from the normal material. The logarithm of the experi-

TABLE 1.—*Distribution of Diabetic Groups According to Age at Death*

Age, Yr.	Males	Females	Normotensive Group	Hypertensive Group
30-39	1	1	1	1
40-49	2	..	..	2
50-59	4	2	..	6
60-69	4	6	..	10
70-79	6	6	3	9

TABLE 2.—*Duration of Diabetes Mellitus*

Recently Diagnosed	Less Than 5 Yr.	5 to 10 Yr.	More Than 5 Yr.	Unknown
3	4	3	14	8

mental value was found, and the deviation of the experimentally determined logarithm from the logarithm calculated from the normal material was used for the evaluation. The influence of age can in this way be excluded from the comparisons.

RESULTS

Dry Weight.—The dry weights of the aortas of the four normotensive and the 27 hypertensive diabetics were known. The mean of the deviations from the normal values were calculated according to the following formula:

$$\text{Log weight} = 0.3171 + 0.0100 \times \text{age.}$$

The results are given in Table 3. It will be seen that the dry weight of the aorta of the normotensive diabetic does not deviate significantly from that of the aorta of the normal normotensive control. In comparison with the hypertensive control group, however, there is a significant difference. In the hypertensive group the diabetics differ significantly in this respect from the normotensive controls, but not significantly from the hypertensive controls. In the hypertensive group the mean

4. Zeek, P. M.: Heart Weight: I. The Weight of the Normal Human Heart, Arch. Path. 34:820 (Nov.) 1942.

deviation from the calculated normal value was lower than the mean of the deviations found in the hypertensive controls.

Cholesterol.—The total cholesterol contents of the aortas of the four normotensive and the 27 hypertensive diabetic patients were known. The expected normal value was calculated according to the following formula:

$$\text{Log cholesterol} = 0.9792 + 0.0241 \times \text{age}.$$

The statistical treatment of the difference from the calculated normal shows results comparable to those obtained in the case of the dry weight. The normotensive diabetic group shows values identical with the normotensive controls, but the significance of the difference from the hypertensive controls was not as great as it was in the case of the dry weight. In the hypertensive group the results are very significant. The diabetic hypertensive group showed identity with the hypertensive controls, and a significant difference from the normotensive controls. In this case,

TABLE 3.—*Deviations of the Aortas of the Diabetic Groups as Compared with Those of the Nondiabetic Controls*

Point of Comparison	Blood Pressure Group	Mean Deviations: Control Group	Mean Deviations: Patients with Diabetes Mellitus	Probability for Difference of Diabetic Vessels from: Normotensive Controls	Probability for Difference of Diabetic Vessels from: Hypertensive Controls
Dry weight	Normotensive	0.0044 ± 0.0130	0.0160 ± 0.013	< 10%	90% < P < 99%
	Hypertensive	0.1225 ± 0.0104	0.1060 ± 0.0223	> 99.9%	< 10%
Total cholesterol	Normotensive	0.0017 ± 0.0131	0.0102 ± 0.0096	< 10%	95% < P < 99%
	Hypertensive	0.3769 ± 0.0099	0.3129 ± 0.0294	> 99.9%	< 10%
Total calcium	Normotensive	0.0163 ± 0.0200	0.0867 ± 0.1217	< 10%	< 10%
	Hypertensive	0.2082 ± 0.0275	0.2932 ± 0.0728	> 99.9%	< 10%

also, the difference from the calculated normal was smaller for the hypertensive diabetic than for the hypertensive control material.

Calcium.—The number of calcium analyses was somewhat smaller than was the case for the two other factors studied. Only three aortas of normotensive and 18 of hypertensive diabetic patients were studied. The normal values for the individual vessels were calculated according to the following formula:

$$\text{Log Ca} = 0.3060 + 0.0318 \times \text{age}.$$

The results agree with the preceding results. However, the normotensive group is too small to demonstrate statistically significant variation. The hypertensive diabetics deviate significantly from the normotensive controls, and show identity with the hypertensive controls. The mean of the calculated deviations for the hypertensive diabetics, however, is somewhat higher than the mean for the hypertensive controls.

COMMENT

The results indicate that the aorta does not participate in the increased vascular sclerosis commonly found in diabetic individuals but follows the behavior of the aorta of the nondiabetic person. This means that the factors determining the development of aortic sclerosis are age and hypertension, and the hypertension which may occur as a complication to the diabetes will be of more significance for the development of sclerosis in the aorta than the diabetes itself.

The findings of Ackerman, Dry, and Edwards [5] for the coronary arteries in some measure support the findings for the aorta. When the grade of coronary sclerosis in the diabetic group is compared with that in the hypertensive group, practically identical figures are found. Taking into consideration that practically all these diabetics will have shown hypertension this can be interpreted only as showing that the diabetics do not have increased coronary sclerosis.

In discussions of the diabetic vascular sclerosis the hypercholesteremia of diabetes is frequently stressed as an accelerating factor. If this should be the case, an increase of the total cholesterol of the aorta should be found. This, however, was not the case. No explanation can be offered for this discrepancy. It has been suggested that the cholesterol deposition seen in the aorta should be dependent on two interacting factors: a lipid factor, represented by the level of serum cholesterol, and a tissue factor, which partly depends on the influence of the blood pressure,[6] especially when hypertension is present. To state that the serum cholesterol level is an indication of the lipids offered for deposition is probably an oversimplification.

In experimental atherosclerosis, findings similar to those reported here have been brought forward from two sides.

McGill and Holman [7] have shown that the hypercholesteremia produced by feeding cholesterol to rabbits did not give rise to cholesterol deposits in the aorta if the rabbit had been made diabetic with alloxan before the feeding was started. This was the case, although serum cholesterol reached somewhat higher levels in the diabetic animals than in the controls, where marked atheromatosis was found.

Another form of hormonally controlled hypercholesteremia apparently shows the same relationship. Chaikoff and associates [8] showed that diethylstilbestrol treatment and cholesterol feeding both gave rise to atheromatosis in the chicken. These forms of inductions, however, gave different types of depositions, and although the serum cholesterol levels reached were the same, the amount of cholesterol deposited in the diethylstilbestrol-treated birds was less.

The hormonally controlled hypercholesteremia of myxedema, however, seems to give rise to cholesterol depositions comparable to those found in xanthomatosis.[9]

Calcification plays an important role in the sclerosis of the peripheral arteries in diabetes mellitus. Roentgenologic and postmortem studies indicate [10] that this calcification spreads to the larger arteries at a much earlier age in diabetic patients than is seen normally. In this connection the aorta can be considered as consisting

5. Ackerman, R. T.; Dry, T. J., and Edwards, J. E.: Relationship of Various Factors to the Degree of Coronary Atherosclerosis in Women, Circulation **1**:1345, 1950.

6. Faber, M.: The Human Aorta: Sulfate-Containing Polyuronides and the Deposition of Cholesterol, Arch. Path. **48**:342 (Oct.) 1949.

7. McGill, H. C., and Holman, R. L.: The Influence of Alloxan Diabetes on Cholesterol Atheromatosis in the Rabbit, Proc. Soc. Exper. Biol. & Med. **72**:72, 1949.

8. Chaikoff, I. L.; Lindsay, S.; Lorenz, F. W., and Entenman, C.: Production of Atheromatosis in the Aorta of the Bird by the Administration of Diethylstilbestrol, J. Exper. Med. **88**:373, 1948.

9. Merkel, H.: Ueber Gefässveränderungen bei dystrophischen Hypothyreoidismus, Beitr. path. Anat. **104**:332, 1940.

10. Peterson, G. F.: Röntgenologische Untersuchungen über Arteriosclerose, Acta radiol. Supp. 39, 1941. Sappington, S. W., and Fisher, H. R.: Arteriosclerosis Obliterans: A Study of the Lesions in Occluding Peripheral Sclerosis, with a Note on Mönckeberg's Sclerosis, Arch. Path. **34**:989, 1942.

of two parts. The lower one-third will behave like the iliac artery, while the upper two-thirds will behave like an elastic artery. It should therefore be possible for the calcification to rise from the extremities to the lower third of the aorta at a time when only minor sclerotic changes can be demonstrated in the upper two-thirds. If this is the case, the mean deviation of the calcium determinations on the diabetic aortas should deviate from the normal more than was the case with the control group. At the same time the dry weight and the cholesterol content, which has to be regarded as a measure of the typical sclerotic lesion of the upper two-thirds, should follow the control group. In this material these calculations can be performed only in the hypertensive group, the normotensive group being too small.

The mean deviations of the aortas of the diabetic hypertensive group follow these lines. In the case of the dry weight and the cholesterol content the deviations are somewhat lower than the means found for the controls, and the deviation for the calcium content is somewhat higher. None of these deviations from the hypertensive controls are statistically significant. When the deviation for the calcium is calculated as if the calcium increase was of the same size as the deviation for the dry weight the expected deviation should be + 0.1650 instead of the experimentally determined + 0.2932. These figures, however, differ less than two times the standard deviation of the mean of the diabetic hypertensive group, which was found to be 0.0728, and the difference cannot be considered significant.

SUMMARY

The dry weight, the total cholesterol, and the total calcium were determined in the intima and the media of the aortas of 32 diabetic persons, four of whom had normal blood pressure and 28 hypertension.

When the figures were compared with those for nondiabetic normotensive and hypertensive persons, it was found that the aortas of the diabetic groups quantitatively followed those of the nondiabetic group. This indicates that the diabetic state is without influence on the arteriosclerosis of the aorta, and that the determining factors are age and blood pressure.

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PATHOGENESIS OF STREPTOCOCCAL PNEUMONIA IN THE RAT

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With the Technical Assistance of Alice Hamlin
ST. LOUIS

AS THE first step in a study of certain aspects of Group-A beta-hemolytic-streptococcus infections in experimental animals, the pathogenesis of streptococcal pneumonia was investigated; the resulting observations form the basis of this report.

In recent years similar observations regarding pneumonia due to the pneumococcus and to Friedländer's bacillus have been described.[1] It has been shown that extension of both pneumococcal and Friedländer pneumonia is due principally to spread of infected edema fluid which carries large numbers of bacteria to uninvolved alveoli. Likewise, edema fluid of the large bronchi presumably constitutes the means by which other lobes become infected.

In humans "primary" pneumonia due to the hemolytic streptococcus is relatively uncommon, accounting under ordinary circumstances for less than 5% of all cases[2]; on the other hand, as a complication of other diseases, especially measles and influenza, it has been widespread in occurrence and was identified with a relatively high mortality rate prior to the advent of chemotherapy.[3]

Dr. Glaser was National Research Council Fellow in the Medical Sciences when this work was done.

From the Infectious Disease Division of the Department of Medicine, and the Oscar Johnson Institute for Medical Research, Washington University School of Medicine.

1. (*a*) Wood, W. B., Jr.: J. Exper. Med. **73**:201, 1941. (*b*) Wood, W. B., Jr., and Irons, E. N.: Ibid. **84**:365, 1946. (*c*) Wood, W. B., Jr.; McLeod, C., and Irons, E. N.: Ibid., p. 377. (*d*) Wood, W. B., Jr.; Smith, M. R., and Watson, B.: Ibid., p. 387. (*e*) Sale, L., Jr., and Wood, W. B., Jr.: Ibid. **86**:239, 1947. (*f*) Sale, L., Jr.; Smith, M. R., and Wood, W. B., Jr.: Ibid. **86**:249, 1947. (*g*) Smith, M. R., and Wood, W. B., Jr.: Ibid. **86**:257, 1947.

2. Heffron, R.: Pneumonia with Special Reference to Pneumococcal Lobar Pneumonia, New York, the Commonwealth Fund, 1939. Keefer, C. S.; Rantz, L. A., and Rammelkamp, C. H., Jr.: Ann. Int. Med. **14**:1533, 1941.

3. Opie, E. L.; Blake, F. G.; Small, J. C., and Rivers, T. M.: Epidemic Respiratory Disease: The Pneumonias and Other Infections of the Respiratory Tract Accompanying Influenza and Measles, St. Louis, C. V. Mosby Company, 1921. MacCallum, W. G.: The Pathology of the Pneumonia in the United States Army Camps During the Winter of 1917-1918, New York, Monograph 10, Rockefeller Institute for Medical Research, April, 1919.

A number of attempts to produce experimental streptococcal pneumonia has been recorded [4]; several of these undertakings were unsuccessful,[5] and in most of the others pathologic observations were limited. Since no systematic description of the course and the histopathologic aspects of experimental streptococcal pneumonia has been published, it was believed desirable to undertake a detailed study of its pathogenesis.

METHODS

In general the techniques employed were those previously described.[1a] Pure strain albino rats, weighing 175 to 250 gm. and free from pulmonary disease, were used in all experiments.

Two strains of Group-A beta hemolytic streptococci were supplied by Dr. Maclyn McCarty of the Hospital of the Rockefeller Institute for Medical Research. These strains were identified as IRS-100 (type 26) and GL-23 (type 17); both were isolated from patients during the acute phase of streptococcal infections.

Although no attempt was made to increase the virulence of either strain to a maximum, each was passed serially through mice (Strain IRS-100 thirteen times and Strain GL-23 eight times)[6]; thereafter, 0.2 ml. of a four-hour culture in beef-infusion broth with 10% sheep serum and 1% dextrose when inoculated intraperitoneally was found to kill mice in 18 to 24 hr. Each strain was stored in defibrinated rabbit's blood at 4 C., or else as a concentrated 18-hr. culture, resuspended in small amounts of fresh serum broth and stored in small tubes at —72 C. The latter procedure has been found to constitute a satisfactory method by which streptococci may be kept for long periods without loss of virulence.[7]

For inoculation of animals, the given strain was grown in 5 ml. of serum dextrose broth at 37 C. for 18 hr.; 0.5 ml. of the 18-hr. culture was transferred to 5 ml. of fresh medium and incubated at 37 C. for four hours.[8] The four-hour culture was then centrifuged for 30 min. at 3,000 revolutions per minute (rpm) in a refrigerated centrifuge—an angle head was used and the temperature within the chamber was maintained at 6 C. The supernatant fluid was removed and the original volume restored with cold beef-infusion broth; after serial tenfold dilutions had been made in cold broth, colony counts were determined on 1-ml. aliquots from the 10^{-7} and 10^{-8} dilutions.

Rats were anesthetized with ether and inoculated with 0.1 ml. of a suspension of streptococci in 6% sterile mucin. This volume of inoculum, which contained 8 to 12 thousand chains of streptococci, was introduced into the left main bronchus. After inoculation the animals were suspended by the upper incisors and kept under light ether anesthesia for 30 min. to insure that the inoculum penetrated the base of the lung.

Twenty animals were inoculated with each strain, and the course of the untreated infection was observed; at the end of two weeks surviving animals were killed and autopsies performed.

In order that the evolution of the pulmonary lesion produced by each strain might be studied, animals were inoculated and killed in groups at 6, 12, 18, and 24 hr., respectively. Blood specimens were obtained for cultures at the time the animals were killed. The gross appearance of each lesion was noted, and the presence or the absence of pleural effusion, pericarditis,

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4. (*a*) Gay, F. P., and Stone, R. L.: J. Infect. Dis. **26**:265, 1920. (*b*) Leake, C. D., and Brown, T. K.: J. Exper. Med. **36**:661, 1922. (*c*) Terrell, E. E.; Robertson, O. H., and Coggeshall, L. T.: Ibid. **12**:393, 1933. (*d*) Wollstein, M., and Meltzer, S. J.: Ibid. **16**:126, 1912; Proc. Soc. Exper. Biol. & Med. **16**:40, 1918. (*e*) Graham, E. A., and Bell, R. D.: Am. J. M. Sc. **156**:839, 1918. (*f*) Blake, F. G., and Cecil, R. L.: J. Exper. Med. **32**:401, 1920. (*g*) Gay, F. P., and Rhodes, B.: J. Infect. Dis. **29**:217, 1921. (*h*) McCordock, H. B., and Muckenfuss, R. S.: Am. J. Path. **9**:221, 1933.

5. Gay and Stone.[4a] Leake and Brown.[4b] Terrell and others.[4c]

6. Subsequently Strain GL-23 attained far greater virulence; it then produced more fulminating infections, which were uniformly fatal (Glaser, R. J.; Berry, J. W.; Loeb, L. H., and Wood, W. B., Jr.: J. Lab. & Clin. Med., to be published).

7. McCarty, M.: Personal communication to the authors.

8. Preliminary observations showed that in a four-hour culture, prepared as described, the streptococci were maximally encapsulated.

empyema, and parenchymal abscesses was recorded. Impression smears were made from longitudinal sections taken through the lesions and were stained with methylene blue.[9] The lungs from at least two animals killed at each interval were fixed in Zenker-formaldehyde solution according to Loosli's[10] method, and sections 7 μ thick were stained by the Gram-Weigert technique. Several such experiments were performed; the observations reported constitute a summary of the group.

RESULTS

Mortality Rate.—In a group of 20 animals pneumonia was produced with Strains IRS-100, and in a group of 21 animals Strain GL-23 was employed. The

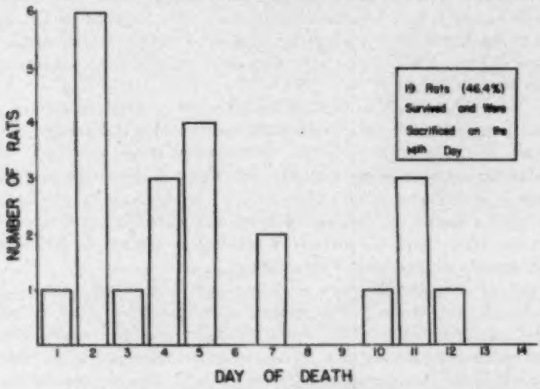

Fig. 1.—Rate at which animals succumb to experimental Group-A streptococcal pneumonia.

TIME AFTER INOCULATION	6 HOURS	12 HOURS	18 HOURS	24 HOURS	30 HOURS
APPROXIMATE SIZE OF PULMONARY LESION					
NUMBER OF ANIMALS	9	9	15	11	3
NUMBER WITH POSITIVE BLOOD CULTURE	0	0	2	3	3
INCIDENCE OF BACTEREMIA (%)	0	0	13	272	100

Fig. 2.—Size of representative untreated lesions at six-hour intervals following induction of Group-A streptococcal pneumonia.

fatality rates were essentially identical, being 55 and 52%, respectively. Figure 1 shows the rate at which animals succumbed to the infection during the two-week period of observation.

Gross Pathologic Observations.—The progression of the pneumonic process, observed at six-hour intervals, is illustrated in Figure 2; the size of representative

9. Methylene blue was employed because it was found to stain the streptococcal capsule metachromatically just as it has been observed to do with the capsule of Type 3 pneumococcus (Wood, W. B., Jr., and Smith, M. R.: J. Exper. Med. **90**:85, 1949).

10. Loosli, C. G.: Arch. Path. **24**:743 (Dec.) 1937.

lesions of a large group of animals and the incidence of bacteremia at given intervals are shown. There was no significant over-all difference in the size of lesions produced by the two strains at any given interval, although with both there were noted variations in the severity of the pneumonia and in the development of complications.

At six hours the involved area of the lung was small and hemorrhagic; its border was usually irregular. Six hours later the lesion had increased in size, with an easily discernible, hemorrhagic, advancing edge, darker than the older portion, which often by 12 and almost always by 18 hr. had taken on a brown or tan color. Maximum involvement could not be predicted for any given time. In the animals which died during the first 72 hr. after inoculation, advanced consolidation was the typical finding, but in those succumbing during the 5th to 12th days, abscess formation, pericarditis, and empyema were commonly observed. Survival to 14 days did not always indicate uneventful recovery. Indeed, autopsies performed on the surviving animals showed that more than half had significant residual lesions. These data are summarized in the table.

Incidence of Bacteremia.—Bacteremia was not observed during the first 12 hr. following inoculation; positive blood cultures were obtained in approximately 13% of the animals at 18 hr. and in 27% at 24 hr.

Autopsy Findings in Nineteen Rats Surviving Fourteen Days After Induction of Streptococcal Pneumonia

Finding	Animals
Empyema	2
Unresolved pneumonia	2
Abscesses	8
Resolved or resolving pneumonia	7
Total	19

Microscopic Pathologic Observations.—Microscopic sections obtained at six-hour intervals revealed the lesion to be essentially alveolar, similar to that of experimental pneumococcal and Friedländer-bacillus pneumonia. At six hours the involved area was small and the cellular reaction limited; a polymorphonuclear leucocytic exudate was present and usually, at the outer edge, some edema fluid containing streptococci was seen (Fig. 3*A*). Even at six hours streptococci were being phagocytosed by the polymorphonuclear leucocytes (Fig. 3*B*).

At 12 hr. the size of the pneumonic lesion was increased, and the process was characterized by a prominent exudative response involving polymorphonuclear leucocytes (Fig. 3*C*). In the oldest portion of the lesion the phagocytosis of streptococci was striking, many leucocytes containing large numbers of organisms. Again at the periphery a zone of edema with free streptococci was noted.

After 18 hr. there was further increase in the extent of pneumonia; in the oldest part of the lesion streptococci were few or absent, whereas near the periphery many were visible, and phagocytosis of the cocci was active. At the edge of the lesion a spreading edematous zone with free cocci was noted (Fig. 3*D*). Exudate containing streptococci was present in the bronchial secretion (Fig. 3*E*), and that the organisms would be conveyed by this route to new areas of the lung could be presumed.

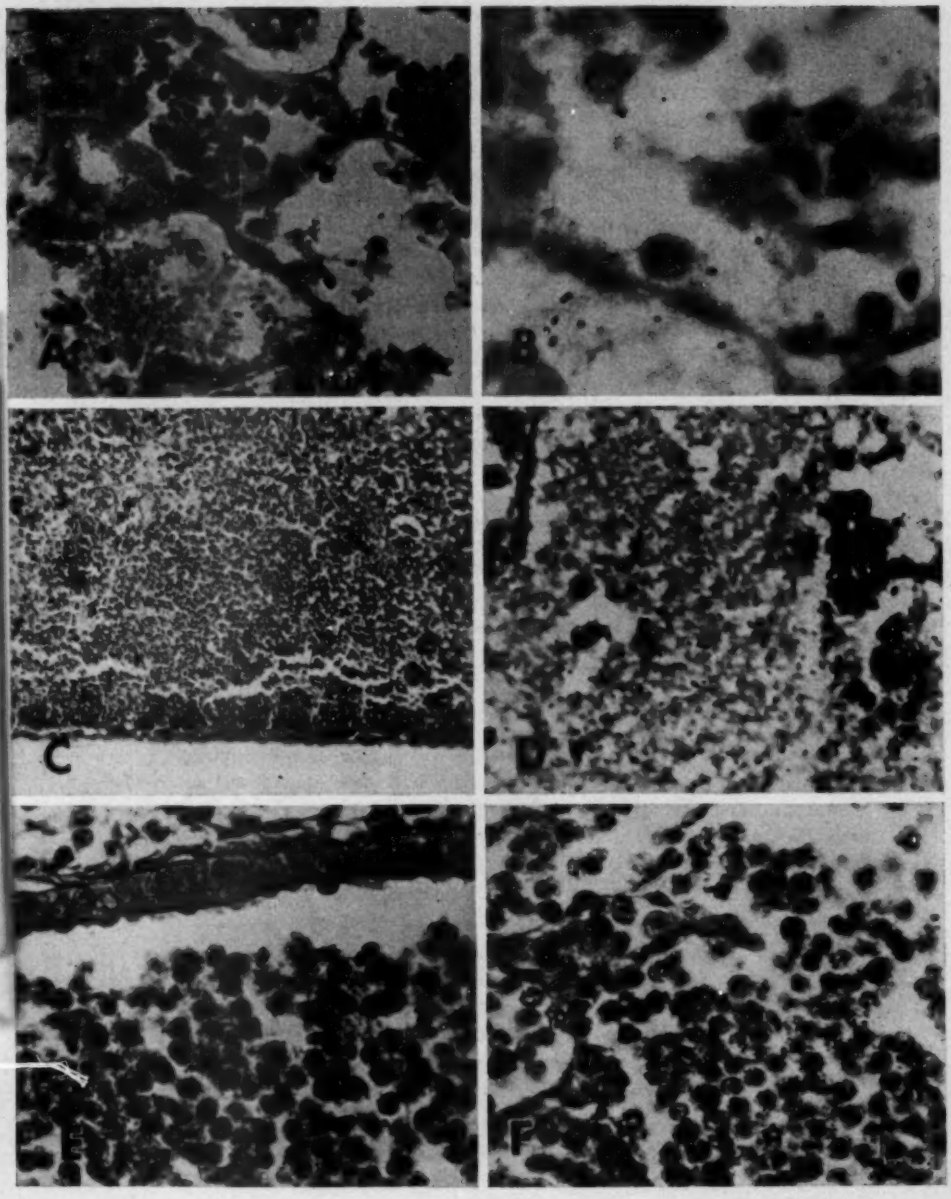

Figure 3

(See legends on opposite page)

In specimens obtained at 24 hr. or later the process was essentially as described for 18 hr. except that in some instances further spread was not obvious; in such animals the spread appeared to have been controlled by the leucocytes, which had phagocytosed all of the organisms (Fig. 3*F*). Macrophages were seen especially in the older areas of the lesion. Fibrin strands were also prominent (Fig. 4*A*). Similarly at 30 hr. an edematous zone with free streptococci was found in some animals, whereas in other specimens the lesion appeared to have been controlled. In animals dying at 42 hr. the pneumonic lesion occasionally showed myriads of streptococci (Fig. 4*B*).

Impression smears of lesions at the various intervals studied showed that in areas where the infection was spreading, and in pleural and pericardial fluid (Fig. 4*C*), the streptococci were encapsulated. Encapsulated organisms were also observed within leucocytes. In vitro studies, employing previously described techniques,[1d] demonstrated "surface phagocytosis" of encapsulated streptococci.

Where control of the infection was not achieved promptly, pericarditis, empyema, or abscesses resulted; when bacteremia developed, the animals died In an occasional specimen early necrosis of pulmonary tissue was noted at 18 hr., although abscess formation did not become evident usually until 24 or 30 hr. (Fig. 4*D*).

Abscesses varied in size; some were very large (Fig. 4*E*), whereas others were relatively small. Often no organisms could be visualized within them in microscopic sections, and in a few instances cultures of the lesions revealed no streptococci.

An interesting incidental finding was the presence of many eosinophils in certain portions of the lesion; particularly about blood vessels tremendous numbers were often present (Fig. 4*F*).

COMMENT

Streptococcal pneumonia produced experimentally in the rat differs considerably in its histopathology from the disease usually described in humans. In the rat the pneumonia is alveolar in character and is thus similar to that caused by pneumococcus and Friedländer's bacillus both in patients and in experimental animals.[11]

11. (*a*) McDermott, W., in Nelson Loose-Leaf Medicine, New York, Thomas Nelson & Sons, 1947, Vol. 1, Chap. 6; (*b*) Vol. 1, Chap. 22.

Explanation of Figure 3

Microscopic sections from the lungs of rats with experimental Group-A streptococcal pneumonia. All of the photomicrographs in Figures 3 and 4 were taken by Mr. K. Cramer Lewis, Department of Illustration, Washington University School of Medicine.

A, six-hour lesion showing edema fluid with streptococci and cellular exudate in the alveoli. Stained by Gram-Weigert technique; × 600.

B, leukocytic phagocytosis of streptococci six hours after intrabronchial inoculation. Stained by Gram-Weigert technique; × 1,530.

C, abundant leukocytic response twelve hours after the inception of pneumonia. The pleural surface is at the lower edge of the section. Stained by Gram-Weigert technique; × 180.

D, edematous zone with many streptococci at the advancing edge of an 18-hr. lesion. Stained by Gram-Weigert technique; × 600.

E, leucocytic exudate and free streptococci in the lumen of a bronchus. Stained by Gram-Weigert technique; × 600.

F, section of a 24-hr. lesion. Although streptococci are numerous, they are all within the leucocytes and the infection is controlled. Stained by Gram-Weigert technique; ×600.

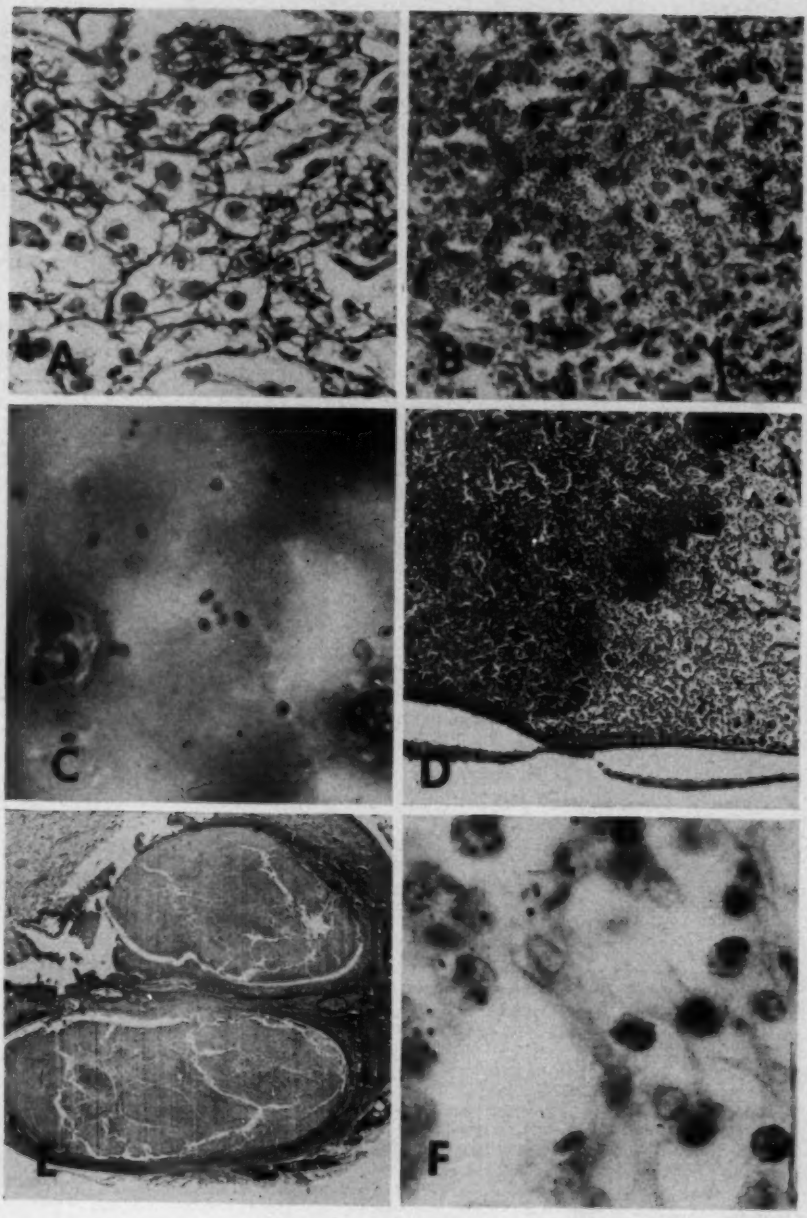

Figure 4

(See legends on opposite page)

On the other hand, most of the descriptions of the histopathologic aspects of human streptococcal pneumonia indicate that the characteristic finding is interstitial pneumonia (bronchopneumonia),[8] although some clinical reports describe "lobar pneumonia" due to hemolytic streptococcus.[12]

The disparate findings reported for human infections and for those observed in this study may possibly be reconciled by a consideration of the fact that primary streptococcal pneumonia is uncommon in man, especially when compared with the so-called secondary form which complicates epidemic influenza and measles. The infrequency of primary streptococcal pneumonia in man as compared with pneumococcal pneumonia is attributed to more effective natural defenses against the former.[11b] The classic histopathologic descriptions of human streptococcal pneumonia have been based on the study of lungs from patients dying of the secondary form,[8] and it is well known that prior damage to the respiratory tract modifies the nature of the lesion caused by a given organism.[4b] Limited observations in two experimental studies [13] suggest that the so-called "lobular pneumonia" produced in dogs and rabbits by intratracheal inoculation of streptococci was not unlike the pneumonia observed in this study.

The mechanism by which the pneumonic lesions due to pneumococcus and to Friedländer's bacillus spreads through the lung has been thoroughly discussed,[14] and the older concept of lymphatic spread [15] has been refuted.[16] The situation which obtains in experimental streptococcal pneumonia is analogous. Spread of the infection results primarily from the fact that edema fluid within alveoli at the outer edge of the lesion and within bronchi affords a means by which organisms are carried to uninvolved tissue. Although the prominence of serous exudation in experimental streptococcal pneumonia was noted as early as 1912,[4d] its significance as regards spread of the lesion was not appreciated.

In the pneumonia produced in this study phagocytosis was often marked in the area of early consolidation. Experimental pneumococcal and Friedländer-bacillus

12. Cecil, R. L.; Baldwin, H. S., and Larsen, N. P.: Lobar Pneumonia: Clinical and Bacteriologic Study of 2,000 Typed Cases, Arch. Int. Med. **40**:253 (Sept.) 1927. Cecil, R. L.: M. Clin. North America **2**:567, 1918-1919.

13. Graham and Bell.[4c] McCordock and Muckenfuss.[4b]

14. Wood.[1a] Sale and Wood.[1c]

15. Blake, F. G., and Cecil, R. L.: J. Exper. Med. **31**:445, 1920.

16. Loosli, C. G.: J. Exper. Med. **76**:79, 1942. Sale and Wood.[1c]

EXPLANATION OF FIGURE 4

A, large amounts of fibrin in a 24-hr. lesion. Stained by Gram-Weigert technique; × 600.

B, fatal lesion (death at 42 hr.) with myriads of streptococci. Stained by Gram-Weigert technique; × 600.

C, encapsulated streptococci in pericardial fluid of an animal killed on the 14th day after inoculation. Fixed smear stained with methylene blue; × 1,800.

D, large abscess in an animal which was infected 30 hr. before; the line of demarcation between abscess and the remaining infected but viable tissue is easily identified. Stained by Gram-Weigert technique; × 60.

E, two huge abscesses in the lung of an animal which survived the infection and was killed on the 14th day. Stained by Gram-Weigert technique.

F, eosinophilic leucocytes in the adventitia of a blood vessel within the pneumonic lesion. Stained by Gram-Weigert technique; × 1,530.

pneumonia by contrast were characterized by little or no phagocytosis in comparable regions.[14] Further, in the present investigation bacteremia occurred later and less often, and the mortality rate was lower, than in the previous studies. It is probable that all of these differences may be explained by the fact that the streptococci employed were relatively less virulent for the rat than were the pneumococci and Friedländer's bacilli used.

Abscess formation occurred in the lungs of a considerable number of rats as a consequence of the streptococcal infection; indeed, significant necrosis of pulmonary tissue was noted as early as 18 hr. after the onset of the pneumonia. The necrotizing character of the Group-A streptococcal infections of humans is well known,[17] and the occurrence of lung abscesses might be expected on this basis alone. Whether the destruction of pulmonary tissue is due solely to a necrotoxin originating from the streptococcus remains to be determined. The excessive numbers of streptococci present in certain parts of the pneumonic lesion suggest that the high density of the bacterial colonization of the tissues may itself contribute to suppuration, just as it appears to do in the case of lung abscesses resulting from pneumonia due to Friedländer's bacillus and pneumococcus Type 3.[18] There is no evidence that the hyaluronic acid of the streptococcal capsule is itself responsible for pulmonary suppuration. Although the capsular hyaluronic acid is the predominant virulence factor for Group-C streptococci,[19] it is considered to be of little importance as far as the virulence of Group-A organisms is concerned.[20]

SUMMARY

Pneumonia due to Group-A beta hemolytic streptococci was induced in albino rats by intrabronchial inoculation. The pneumonic lesion which resulted was lobar in type and caused a mortality rate of slightly over 50%.

The pathogenesis and the course of the pneumonic lesion were studied by examination of serial gross and microscopic sections. Pneumonia due to hemolytic streptococcus was found to resemble closely that produced under similar experimental conditions with pneumococcus and with Friedländer's bacillus. The mechanisms of spread and host defense appeared to be the same as those observed in other forms of acute bacterial pneumonia. Bacteremia, however, occurred later and less frequently, and the mortality was lower than in experimental pneumococcal pneumonia, presumably because the streptococci employed were by comparison less virulent. In spite of their failure to produce uniformly fatal infections, they caused a high incidence of lung abscesses. In the latter respect they simulated Type-3 pneumococcus and Friedländer's bacillus and differed strikingly from pneumococcus Type 1.

Miss Mary Ruth Smith rendered valuable advice and assistance. Mrs. Virgil Loeb Jr. made the charts.

17. Swift, H. F., in Dubos, R. J.: Bacterial and Mycotic Infections of Man, Philadelphia, J. B. Lippincott Company, 1948, Chap. 11.

18. Wood, W. B., Jr., and Smith, M. R.: J. Exper. Med. **92**:85, 1950. Sale and Wood.[16]

19. Hirst, G. K.: J. Exper. Med. **73**:493, 1941.

20. Rothbard, S.: J. Exper. Med. **88**:325, 1948.

EFFECT OF REPEATED STREPTOCOCCAL PULMONARY INFECTIONS ON THE CARDIOVASCULAR SYSTEM IN RATS

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IT IS NOW generally accepted that rheumatic fever develops in man as a sequel to infection with Group-A beta hemolytic streptococci; the pathogenesis of the disease, however, remains obscure. If an identical or, at least, an analogous disease could be produced in experimental animals, it would afford a valuable means of elucidating further the etiology of rheumatic fever. The studies described in this report deal with the effect of repeated streptococcal pulmonary infections on the cardiovascular system of rats.

METHODS

The same techniques as those described in the preceding paper [1] were used to induce repeated attacks of streptococcal pneumonia in albino rats. Young, growing rats,[2] weighing 175 to 200 gm., and free from prior infection, were used in all experiments. Diet consisted of a commercial food [3] and water ad libitum.

Two strains of streptococci (IGL-23 and IRS-100), isolated from patients with acute rheumatic fever, were used as the infecting organisms. A four-hour culture was centrifuged and the supernatant fluid removed; the volume was restored, and tenfold dilutions were made with serum-free broth. The final inoculum, resuspended in 6% mucin, contained 3,000 to 6,000 chains. At the time of each inoculation three or more normal control rats were infected to verify the production of a significant pneumonic lesion.

Eighteen hours after inoculation, the test animals were treated with 6,000 units of penicillin procaine for aqueous injection U. S. P. (aquacillin® [4]) intramuscularly; the same dose was given 24 and 42 hr. after inoculation, and thereafter 3,000 units was injected daily for five days. Under the conditions of the study this therapeutic regimen was demonstrated to result in good resolution of the lesion, usually without complications such as abscess or empyema.

This study was aided by a grant from the Life Insurance Medical Research Fund.

Dr. Glaser was National Research Council Fellow in the Medical Sciences when this work was begun.

Presented at the Twenty-Second Annual Meeting of the Central Society for Clinical Research, Chicago, Nov. 4, 1949.

From the Infectious Disease Division of the Department of Medicine, the Department of Pathology, and the Oscar Johnson Institute for Medical Research, Washington University School of Medicine.

1. Glaser, R. J., and Wood, W. B., Jr.: Pathogenesis of Streptococcal Pneumonia in the Rat, A. M. A. Arch. Path., this issue, p. 244.

2. The rats were obtained from Rockland Farms, New City, N. Y.

3. The food was friskies® made by Albers Milling Company, Peoria, Ill.

4. This preparation was supplied by Mr. C. B. Taft, Schenley Laboratories, Inc., New York.

Forty-four rats were infected at two to three-week intervals. The two strains of streptococci were used alternately, and the inoculum was introduced alternately into the left and the right lung. Preliminary observations indicated that satisfactory lesions could be produced under such circumstances [5]; other findings incident to such repeated infections will be described in a subsequent publication.[6]

The rats were infected two to seven times and then killed. Autopsies were performed without delay. Gross observations were recorded, and the hearts and the kidneys were fixed in Zenker-formaldehyde solution. Microscopic sections were stained with hematoxylin-eosin; in a number of instances Gram-Weigert stains were also employed.

Control histologic observations were made on sections of the hearts from two groups of animals. The first group consisted of 32 normal rats; they were kept under the same environmental conditions as the infected animals and killed at comparable intervals. The second group was designed to control the following specific and nonspecific variables: (1) prolonged penicillin therapy; (2) repeated introduction of mucin into the lung; (3) repeated stress of inhalation anesthesia, and (4) the effect of multiple infections with an organism other than the Group-A beta hemolytic streptococcus. In order to achieve this end, 25 rats were infected at two to three-week intervals with three strains of pneumococci (Type I A5, Type II D-395, and Type III 8HCC), and all other experimental conditions were made to conform exactly with those employed in the streptococcal series.

OBSERVATIONS DURING THE COURSE OF REPEATED INFECTIONS

The rats subjected to multiple pneumonic infections with streptococci or pneumococci exhibited no signs other than those characteristic of the usual reaction to acute bacterial pneumonia.[7] After each infection, the animals responded to chemotherapy and subsequently appeared as well as the uninfected controls; in the occasional animals in which empyema developed, evidence of loss of weight was noted. In none of the animals did arthritis develop.

EFFECTS OF REPEATED INFECTIONS ON THE CARDIOVASCULAR SYSTEM

Gross Pathologic Findings.—Because of the size of the rat's heart, gross observations are of no value in assessing possible changes due to repeated infection. Four animals in the streptococcal group exhibited signs of resolved pericarditis. In the lungs of most of the animals subjected to multiple infections, small fibrous scars were the only evidence of the previous infections. In some animals small chronic abscesses with thick, fibrous walls were noted; occasionally the contents were thick or granular, but frank pus was never observed. Attempts to demonstrate streptococci by smear or culture of abscess contents were unsuccessful.

The kidneys and joints appeared grossly normal as did the other viscera.

Histologic Observations.—The incidence of microscopic lesions in the hearts of animals in each of the three groups studied is shown in the table. The changes were qualitatively similar in all three groups of rats. The appearance of the various lesions was as follows:

(*a*) Myocardium: Two types of alteration were found. The first consisted of small collections of cells distributed perivascularly (Fig. 1*A*). These collections were comprised primarily of lymphocytes, Anitschkow myocytes, and mononuclear mesenchymal cells. The mesenchymal cells were large, with pale, oval or slightly indented nuclei; they contained no nucleoli and little chromatin and had little

5. Glaser, R. J., and Wood, W. B., Jr.: Unpublished observations.
6. Glaser, R. J.; Loeb, L. H., and Wood, W. B., Jr.: To be published.
7. Wood, W. B., Jr.; J. Exper. Med. **73**:201, 1941; Glaser and Wood.[1]

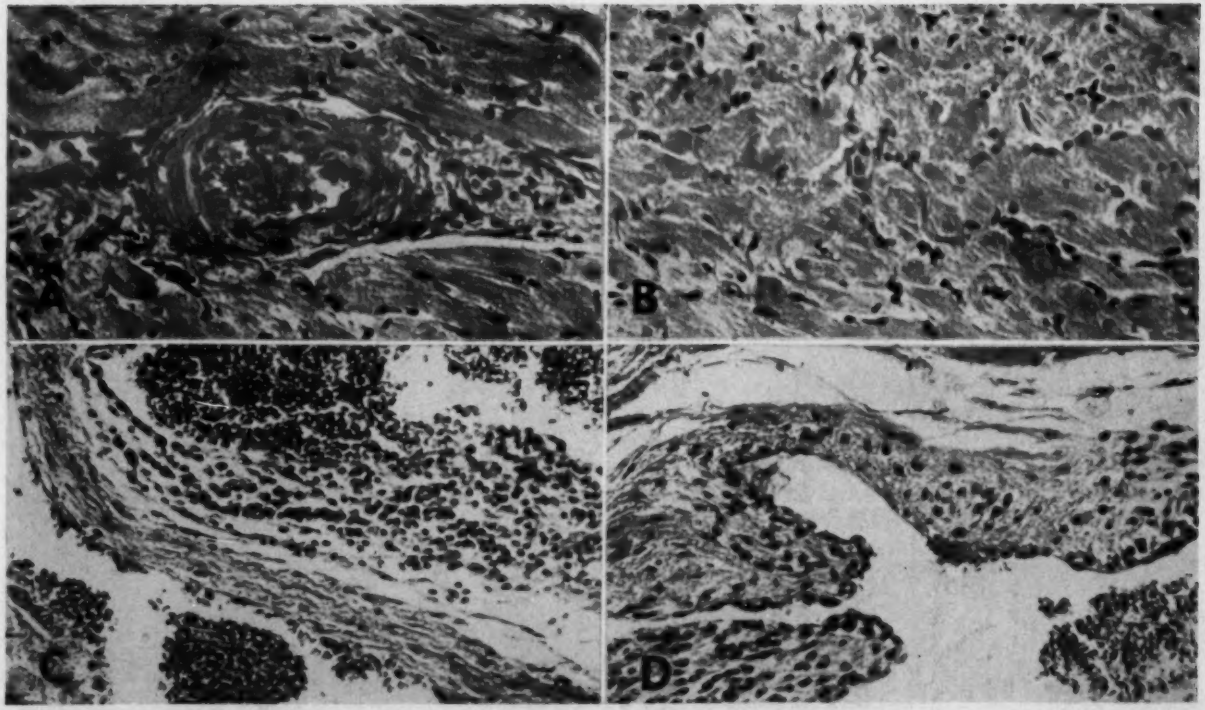

Fig. 1.—This and the following figure present microscopic sections from the hearts of rats subjected to multiple streptococcal infections and from the hearts of normal controls. The photomicrographs were taken by K. Cramer Lewis, Department of Illustration, Washington University School of Medicine.

A, perivascular collection of lymphocytes and mononuclear mesenchymal cells in the myocardium. Hematoxylin-eosin; × 320.

B, linear interstitial infiltrate of lymphocytes and occasional mononuclear mesenchymal cells in the myocardium. Hematoxylin–eosin; × 320.

C, subendocardial collection of lymphocytes, plasma cells, and large mononuclear mesenchymal cells in a mitral valve. Hematoxylin-eosin; × 240.

D, palisade arrangement of large mononuclear mesenchymal cells with occasional fibroblasts, lymphocytes, and Anitschkow cells in a mitral valve. Hematoxylin-eosin; × 240.

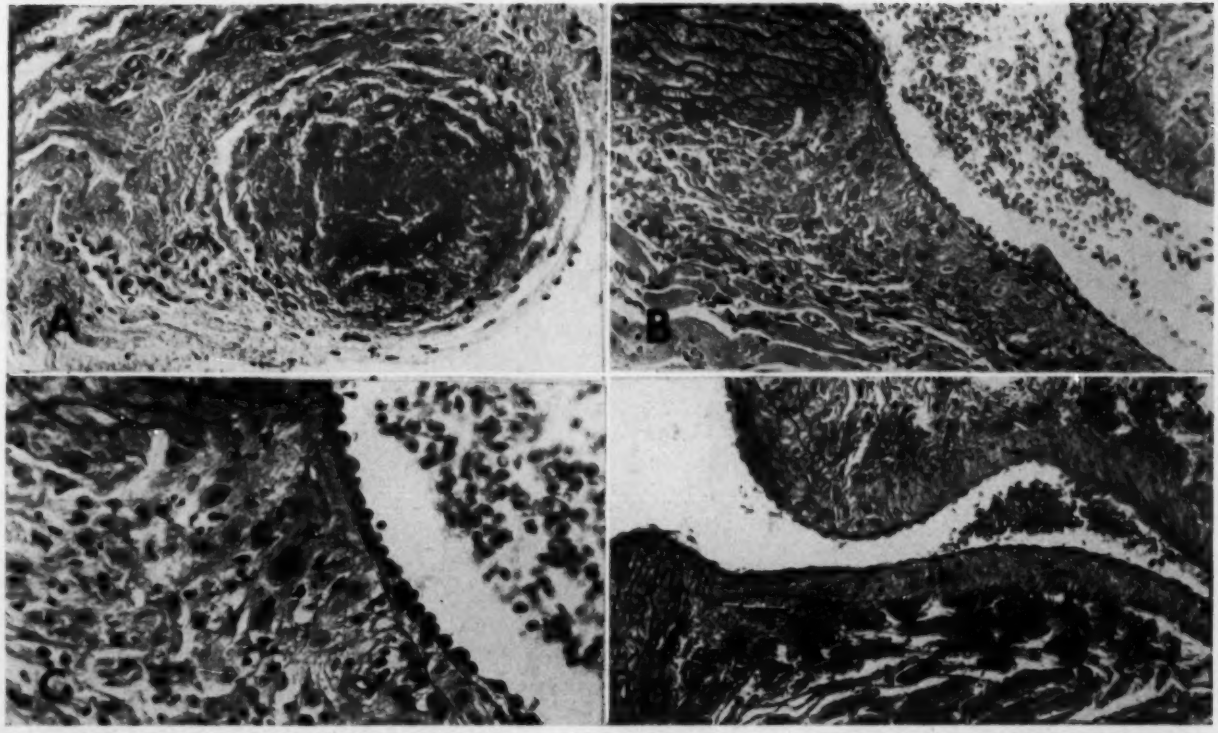

Fig. 2.—*A*, necrosis and fibrinoid deposition with intramural and perivascular infiltrate of mononuclear mesenchymal cells, Anitschkow cells, and lymphocytes in a myocardial artery. Hematoxylin-eosin; × 250.

B, a coronary artery at its origin showing endothelial proliferation, necrosis involving all arterial coats, and an infiltrate of mononuclear mesenchymal cells, Anitschkow cells, and occasional polymorphonuclear leukocytes. Hematoxylin–eosin; × 190.

C, portion of the coronary artery seen in *B*. Hematoxylin-eosin; × 380.

D, normal coronary artery at its origin. Compare with *B* and *C*. Hematoxylin–eosin; × 200.

cytoplasm. Occasional mesenchymal cells contained more chromatin and relatively abundant amphophilic cytoplasm. The second alteration was represented by collections of lymphocytes and mesenchymal cells in the interstitial tissue, distributed in linear fashion without reference to the myocardial arteries (Fig. 1*B*).

The lesions were considered to indicate a slight degree of interstitial myocarditis. There was no alteration in the myocardial fibers or in the collagen, and no granulomas were noted. There were no cells meeting the description of the Aschoff cell, nor did the changes resemble any of the various types of Aschoff bodies.

(*b*) Valves: Lesions of two types were found in the mural and valvular endocardium. The first consisted of subendocardial collections of lymphocytes, plasma cells, mesenchymal cells, and occasional Anitschkow cells (Fig. 1*C*). The second, observed less often, was not as cellular; it presented a palisaded arrangement of mesenchymal cells, fibroblasts, and occasional Anitschkow cells (Fig. 1*D*). No alteration of the collagen, endocardial deposition of fibrin, or increased vascularity of the cusp accompanied either form of valvular lesion, but both types were associated with some degree of thickening of the valve cusps.

At the base of the valves there were occasional cellular foci consisting of Anitschkow cells, mesenchymal cells, and lymphocytes. No collagen alteration or granulomas were observed.

Incidence of Cardiovascular Lesions in Rats Subjected to Repeated Pulmonary Infections and in Normal Controls

Experimental Group	Animals	Incidence of Given Type of Lesion		
		Myocardial	Valvular	Arterial
Streptococcal	44	24 (54.5%)	20 (45.4%)	13 (29.5%)
Pneumococcal	23	8 (34.7%)	13 (56.5%)	2 (8.6%)
Uninfected	32	7 (21.8%)	15 (46.8%)	1 (3.1%)

The aortic and mitral valves were involved with approximately the same frequency. The pulmonary and tricuspid valves were rarely involved.

(*c*) Arteries: The arterial lesions consisted of a segmental panarteritis. In the smaller arteries, deposition of fibrinoid in a necrotic media and cellular infiltration of the adventitia were observed (Fig. 2*A*). In the larger arteries, less fibrinoid was noted, but the cellular infiltration of all the coats was more outstanding. In one of the animals in the streptococcal group, such a lesion was observed in a coronary artery at its origin (Fig. 2*B*); Anitschkow cells were prominent and occasional polymorphonuclear leukocytes were present (Fig. 2*C*). A section of a normal coronary artery at its origin did not show these abnormalities (Fig. 2*D*). Neither verrucous arteritis nor endarteritis polyposa was observed.

Microscopic examination of the kidneys revealed no abnormalities.

COMMENT

That streptococcal infection is related to rheumatic fever in man has been emphasized by the studies of Coburn and others.[8] Since the delineation of the antigenic structure of hemolytic streptococci by Lancefield,[9] the specific importance of infections with Group-A organisms has been appreciated. To explain the patho-

8. Coburn, A. F.: The Factor of Infection in the Rheumatic State, Baltimore, Williams & Wilkins Company, 1931. Sheldon, W.: Lancet **1**:1337, 1931. Schlesinger, B.: Arch. Dis. Childhood **5**:411, 1930. Collis, W. R. F.: Lancet **1**:1341, 1931.

9. Lancefield, R. C.: Harvey Lect. **36**:251, 1940-1941.

genesis of rheumatic fever, hypersensitiveness to the Streptococcus, to its products, or to tissues altered by the Streptococcus has been postulated; this subject has been reviewed recently by Waksman and Fischel.[10]

In planning the study reported herewith, an attempt was made to simulate those conditions under which rheumatic fever develops in man; i. e., infection with Group-A streptococci,[8] involving the respiratory tract,[8] and induced with multiple types at shortly spaced intervals.[11] Because attempts to produce infections of the upper respiratory tract in the rat were unsuccessful,[12] streptococcal pneumonia was the form of infection employed. It should be noted that this lesion, which has been previously described,[1] is more fulminating than the usual streptococcal infection of the upper respiratory tract which is related to the development of rheumatic fever in man. The rat was used in this study with the realization that it responds poorly, if at all, to soluble antigens which are potent stimuli to antibody production in other experimental animals and in man.[13]

The incidence of myocardial and arterial lesions was significantly higher in the animals infected repeatedly with streptococci than in either of the control groups.[14] The myocardial lesion was characterized by interstitial myocarditis of slight degree, less intense than that observed by Wilens and Sproul to occur spontaneously in aged albino rats.[15] The lesion did not resemble that seen in rheumatic myocarditis in man. The spontaneous myocardial lesions observed in mice,[16] which are said to bear some resemblance to rheumatic myocarditis, have been interpreted as occurring on the basis of hypersensitiveness to environmental factors.[16a,b] It may be significant that the mouse, which is more capable of developing immune responses, including anaphylaxis, than is the rat, should show spontaneous development of lesions resembling rheumatic myocarditis, which are not observed in the rat.

The high incidence of arterial lesions in the streptococcal as compared with the pneumococcal and uninfected control groups is more striking. A similar panarteritis, occurring spontaneously and resembling polyarteritis nodosa in man, was observed by Wilens and Sproul, but only in rats over 500 days old.[17] They reported an incidence of 3% in the 500- to 700-day age group, 13.1% in those 700 to 900 days old, and 15.7% in animals over 900 days of age. Such data increase the significance of the lesions observed in our study.

10. Waksman, B. H.: Medicine **28**:143, 1949. Fischel, E. E.: Am. J. Med. **7**:772, 1949.

11. Rantz, C. A.; Boisvert, P. J., and Spink, W. W.: Etiology and Pathogenesis of Rheumatic Fever, Arch. Int. Med. **76**:131 (Sept) 1945.

12. Glaser, R. J., and Loeb, L. H.: Unpublished observations.

13. Longcope, W. T.: J. Exper. Med. **36**:627, 1922. Opie, E. L.: J. Immunol. **9**:231, 1924. Shwartzman, G.: Phenomenon of Local Tissue Reactivity, New York, Paul B. Hoeber, Inc., 1937.

14. Values for χ^2 were calculated from a fourfold table utilizing the correction of Yates for small numbers (Hill, A. B.: Principles of Medical Statistics, ed. 2, London, Lancet, Inc., 1939); values greater than 0.841 are significant, and those greater than 6.635 are highly significant. The statistical data are given in the following tabulation:

Animal Groups	χ^2 Values	
	Myocardial	Arterial
Streptococcal vs. pneumococcal	1.636	2.674
Streptococcal vs. control	4.350	7.098

15. Wilens, S. L., and Sproul, E. E.; Am. J. Path. **14**:177, 1938.

16. (*a*) Gray, F. G.: Am. J. Path. **25**:1215, 1949. (*b*) Moore, F. J.; Ridge, G. K.; Huntington, R. W.; Hall, E. M.; Griffith, G. C., and Knowles, R. G.: Proc. Soc. Exper. Biol. & Med. **65**:102, 1947. (*c*) Lenke, S. E., and Loewe, L.: Am. J. Path. **17**:857, 1941.

17. Wilens, S. L., and Sproul, E. E.: Am. J. Path. **14**:201, 1938.

The incidence of valvular lesions was high in all three experimental groups. In none of the involved valves was deposition of fibrinoid, collagen alteration, endocardial fibrin thrombi, or increased vascularity of the cusps encountered. These features were also lacking in the "rheumatic-like" lesions observed in mice.[16a] That the rat may exhibit fibrinoid under various experimental conditions is apparent from the nature of the arterial lesions observed in the present study as well as in those of other investigators.[18]

The report of Murphy and Swift [19] is of interest in connection with the present investigation; their approach was analogous in that they directed their efforts toward producing rheumatic carditis in rabbits by repeated cutaneous Group-A streptococcal infections. The myocardial lesions they observed were described as "strikingly similar to the most characteristic of all human rheumatic fever lesions, the Aschoff bodies." The valvular lesions also were considered to resemble closely rheumatic endocarditis. Panarteritis of the type observed in our streptococcal group of rats and in rabbits given large amounts of foreign protein [20] was not encountered by Murphy and Swift.

Whether the site of infection of animals is of importance in producing an experimental entity similar to rheumatic fever is not clear. It is of interest, however, that in man nonrespiratory tract infections with a given strain of Streptococcus have not been associated with the development of rheumatic fever whereas rheumatic fever has occurred after respiratory tract infections with the same strain.[21]

SUMMARY

Group-A streptococcal pneumonia was induced two to seven times in albino rats at two to three-week intervals; two strains of streptococci were used alternately and were inoculated first into the left lung and then into the right lung. Repository penicillin therapy was begun 18 hr. after infection and continued for one week. At the conclusion of the course of infections, the animals were killed, and microscopic sections of their hearts were prepared for histologic study. Control observations were made on hearts from rats subjected to multiple infections with several types of pneumococci and on hearts from healthy animals kept under identical environmental conditions.

Interstitial myocarditis and segmental panarteritis occurred more frequently in the animals with streptococcal infections than in either of the two control groups; the difference in incidence was statistically significant. Since valvular lesions occurred in approximately half the animals in each group, they could not be attributed to the repeated infections.

None of the lesions encountered in this investigation resembled any of the various types of Aschoff bodies characteristic of human rheumatic fever.

18. Tong, W. T.: Arch. path. Anat. **300**:373, 1937. Zeek, P. M.; Smith, C. C., and Weeter, J. C.: Am. J. Path. **24**:889, 1948.

19. Murphy, G. E., and Swift, H. F.: J. Exper. Med. **89**:687, 1949.

20. McKeown, E. F.; J. Path. & Bact. **59**:547, 1947. Ehrich, W. E.; Seifter, J., and Forman, C.: J. Exper. Med. **89**:23, 1949. Rich, A. R., and Gregory, J. E.: Bull. Johns Hopkins Hosp. **72**:65, 1943. Dammin, G. J.; Bukantz, S. C., and Alexander, H. L.: Biologic Response in Experimental Hypersensitivity, J. A. M. A. **139**:358 (Feb. 5) 1949. Hopps, H. C., and Wissler, R. W.: J. Lab. & Clin. Med. **31**:939, 1946.

21. Coburn, A. F.: Personal communication to the authors.

EXPERIMENTAL CORONARY SCLEROSIS

IV. The Effect of Cortisone on End-Stage Coronary Sclerosis in Chickens

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IT HAS been shown previously that coronary sclerosis of the "spontaneous" variety is extremely common in adult fowl, that more than 70 per cent of 1-yr.-old birds show some evidence of the disease,[1a,b] and that the sclerosing and stenosing process becomes progressively severer with age.[2] In this naturally occurring form of sclerosis, the later stages are characterized principally by an extensive proliferation of connective tissue cells of the intima, and this leads to narrowing of the arterial lumen, sometimes to a striking degree. A typical example of "spontaneous" coronary sclerosis in an elderly chicken is shown in Figure 1 of this paper.

The beneficial effects of cortisone in rheumatoid arthritis, and its harmful effects in pulmonary tuberculosis, have been laid, in part, to an alleged fibrolytic activity, to a capacity for breaking down and dissolving preformed fibrous tissue. We have investigated this alleged fibrolytic action of cortisone by determining its effect on the number and the size of naturally occurring fibrous plaques of the coronary arteries of elderly chickens.

MATERIAL AND METHOD

After several unsuccessful attempts to find a large enough flock of elderly chickens of the same line, sex, and age and with an adequate amount of "spontaneous" coronary sclerosis, we were finally successful in obtaining an experimental colony that satisfied all requirements.

Fifty 3½-yr.-old white leghorn hens, from the same line and weighing approximately 5 lb. (2 kg.) each, were obtained from Norman F. Schultz, Spruceleigh Farms, Brantford, Ont., Canada. As a preliminary measure, 10 of the hens were killed by electricity, and serial

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From the Department of Medical Research, University of Western Ontario, London, Canada, (Dr. Paterson), and the Division of Animal Pathology, Dominion Deparment of Agriculture, Hull, Province of Quebec (Dr. Mitchell).

1. (*a*) Paterson, J. C.; Slinger, S. J., and Gartley, K. M.: Experimental Coronary Sclerosis: Medical Degeneration as Primary Lesion in Coronary Sclerosis of Cockerels, Arch. Path. **45**: 306 (March) 1948. (*b*) Paterson, J. C.; Mitchell, C. A., and Wallace, A. C.: Experimental Coronary Sclerosis: The Role of Infection in Coronary Sclerosis of Cockerels, ibid. **47**:335 (April) 1949. (*c*) Paterson, J. C., and Cottral, G. E.: Experimental Coronary Sclerosis: III. Lymphomatosis as a Cause of Coronary Sclerosis in Chickens, ibid. **49**:699 (June) 1950.

2. Paterson, J. C., and Gartley, K. M.: Unpublished observations.

sections of their hearts (and coronary artery systems) made. On examining these sections, we found a degree of coronary sclerosis that was severe enough to justify the carrying out of the main experiment.

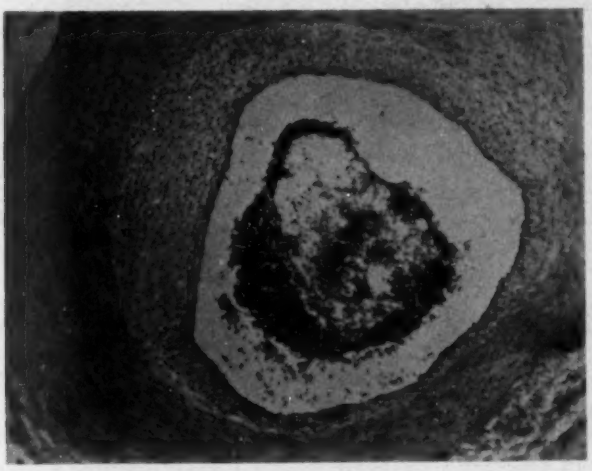

Fig. 1.—An example of advanced coronary sclerosis in a 3½-yr.-old chicken. The arrow points to the internal elastic lamina. Note the extreme grade of fibroblastic proliferation of the intima. Hematoxylin and eosin; × 30.

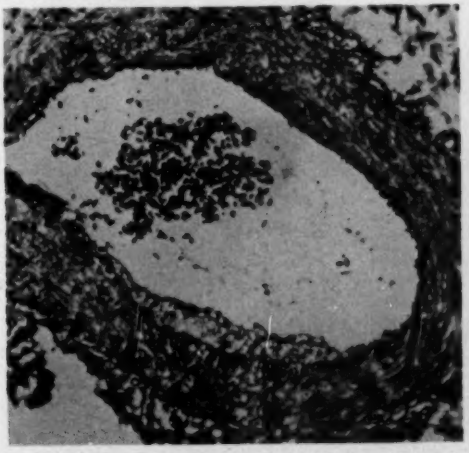

Fig. 2.—Photomicrograph of a coronary artery of a chicken illustrating a slight grade of intimal proliferation. Hematoxylin and eosin; × 100.

The remainder of the series was divided into two groups of 20 hens each. The birds of each group were housed in adjacent pens and fed a commercial growing ration. The Group-1 birds were each given a daily subcutaneous injection of cortisone acetate, at a dosage of 4.5 mg. per kilogram of body weight, and these injections were continued for 14 days. The Group-2 birds, the control group, were not given any treatment. On the 15th day after the first injection

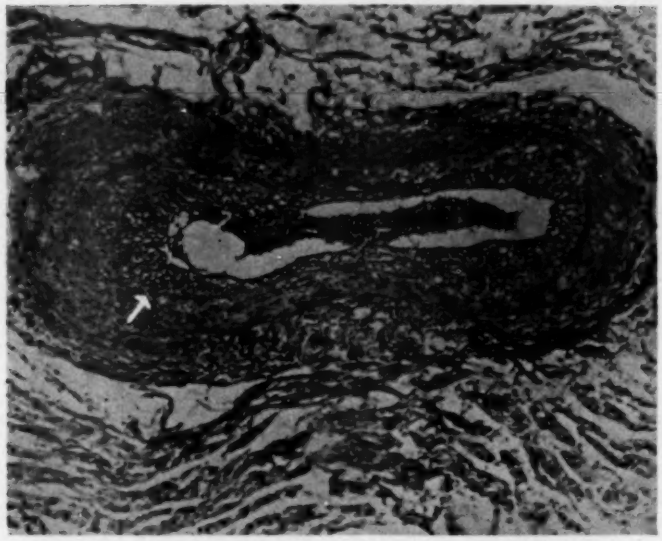

Fig. 3.—Photomicrograph of a coronary artery of a chicken illustrating a moderate grade of intimal proliferation. The arrow points to the internal elastic lamina. Hematoxylin and eosin; × 100.

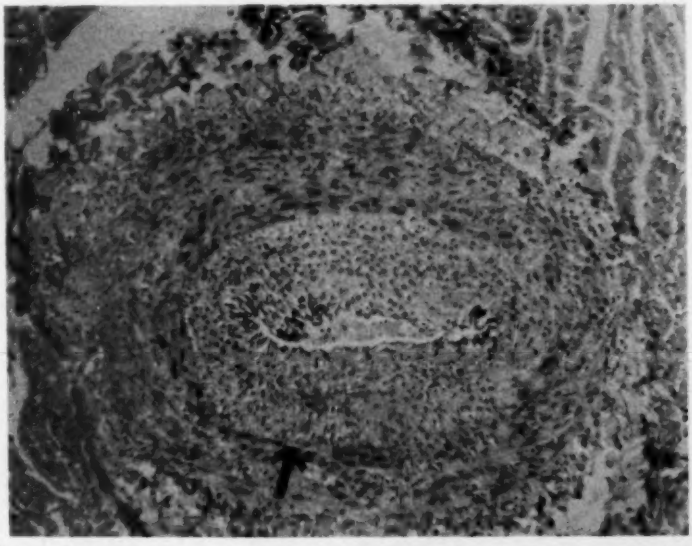

Fig. 4.—Photomicrograph of a coronary artery of a chicken illustrating a marked grade of intimal proliferation. The arrow points to the internal elastic lamina. Hematoxylin and eosin; × 120.

given to the Group 1 birds, all of the hens were killed by electricity. Their hearts were fixed in formaldehyde solution, cut into blocks, and sectioned serially at intervals of 400 μ in the same fashion as in previous experiments.[1] The assessment of the incidence and the degree of coronary sclerosis in each bird was done as follows: Every cross section of a coronary artery measuring more than 250 μ in its widest diameter, more or less, was counted and examined. The incidence of disease was determined by counting the number of arteries with any degree of intimal proliferation and comparing this number with the total number of arteries examined. The degree of disease was determined by assigning a grade to each diseased artery, depending on the thickness of the layer of proliferating tissue. Representative examples of the three grades of intimal proliferation are shown in Figures 2, 3, and 4. As a further check on the assessment by grading, the actual thickness of the intima in each of the lesions in the two groups of birds was measured with a micrometer eyepiece.

TABLE 1.—*Effect of Cortisone on End-Stage "Spontaneous" Coronary Sclerosis of Elderly Chickens*

	Control Birds			Treated Birds		
Serial Number	Number of Arteries Examined	Number of Diseased Arteries	Maximum Degree of Disease	Number of Arteries Examined	Number of Diseased Arteries	Maximum Degree of Disease
1	121	0	0	101	3	+
2	83	46	++++	104	21	++
3	96	2	±	70	37	+++
4	75	2	±	47	21	++
5	73	22	++	54	13	++
6	56	27	++	39	39	++
7	58	0	0	56	0	0
8	49	30	+++	48	18	++
9	89	18	+++	64	0	0
10	67	16	+++	66	0	0
11	56	11	++	50	0	0
12	50	13	+++	70	6	+
13	67	49	++++	70	30	++
14	76	50	+++	82	1	±
15	90	54	++++	72	0	0
16	43	1	+	45	0	0
17	128	9	+	81	0	0
18	81	12	+++	98	4	++
19	71	27	+++	47	1	±
20	102	1	+	76	16	++
Total	1,484	390		1,355	191	

OBSERVATIONS

The number of lesions in the coronary arteries of each bird in the two groups, and the severity of individual lesions, are given in detail in Tables 1, 2, and 3.

The results as summarized in Table 1 show that there were almost twice as many foci of arterial disease in the control group (390 lesions in 1,484 cross sections of arteries, or 26%) as in the cortisone-treated group (191 lesions in 1,355 cross sections of arteries, or 14%). The data on the general incidence of arterial lesions have been subjected to statistical analysis, using the chi-square test, and the difference between the two groups is highly significant (P — less than 0.001).

A difference in the severity of arterial disease in the two groups was more difficult to determine, but by our methods of assessment a marked reduction of the degree of disease was demonstrated as a result of the administration of cortisone. We assessed the severity of disease by grading (Table 2) and by actual measurement (Table 3). In grading the severity of lesions we depended, entirely,

on the judgment of a single observer (J. C. P.) who assigned to each lesion, from its microscopic appearance, one of three grades—slight, moderate, or marked. The method has obvious disadvantages: It was often difficult to assign a grade to a borderline lesion, and when this situation arose, the policy followed was to place the lesion in the grade of less severity. In order to eliminate the element of personal opinion, the lesions were reassessed by measuring their actual maximal thickness with a micrometer eyepiece; but here again there is a fallacy in that the thickness of an intimal plaque may sometimes be exaggerated by tangential cutting.

In spite of the possible errors in our methods of assessment, the results were so impressive and so consistent that one cannot avoid the conclusion that cortisone therapy is followed by an appreciable reduction in the size of "spontaneous" intimal plaques in the coronary arteries of elderly chickens. Lesions of a marked grade

TABLE 2.—*Degree of Intimal Proliferation (by Grading) in Control and Cortisone-Treated Chickens*

	Degree of Intimal Proliferation		
	Slight	Moderate	Marked
Control birds (330 diseased arteries)	140 (30%)	135 (35%)	115 (29%)
Treated birds (191 diseased arteries)	142 (74%)	46 (24%)	3 (2%)

TABLE 3.—*Degree of Intimal Proliferation (by Measurement) in Control and Cortisone-Treated Chickens*

	Degree of Intimal Proliferation		
	Slight (1 to 46 μ Thick)	Moderate (47 to 93 μ Thick)	Marked (More Than 93 μ Thick)
Control birds (330 diseased arteries)	168 (46%)	115 (30%)	92 (24%)
Treated birds (191 diseased arteries)	144 (75%)	40 (21%)	7 (4%)

disappeared almost entirely in the cortisone-treated group, and it is therefore reasonable to assume that moderate-sized plaques were also reduced in thickness and that those of a slight grade were entirely removed, thus accounting for the fact that the number of lesions in the treated group of birds was lower than that in the controls.

COMMENT

The results obtained in this experiment were unexpected. It was not considered possible for any therapeutic procedure, in the short space of two weeks, to affect appreciably the type of fibroblastic proliferation under study. And in this regard it is noted than Spain and his co-workers [3] failed to demonstrate any fibrolytic effect of cortisone on preformed granulation tissue in experimental wounds of mice. Our positive results were even more surprising in that the underlying cause of the fibrosis in the arteries of chickens has been shown previously to be lymphomatosis [1c]—a malignant, infective disease of lymphoid tissue which there is reason to believe is accelerated, not inhibited, by cortisone administration.[4] This inconsistency

3. Spain, D. M.; Molomut, N., and Haber, A.: Biological Studies on Cortisone in Mice, Science **112**:335 (Sept. 22) 1950.

4. Paterson, J. C., and Cottral, G. E.: Unpublished observations.

may be explained, however, by the fact that active "vascular" lymphomatosis is apparent only in young birds; in older chickens, like those used in the present study, the lymphomatous deposits in arterial walls have largely disappeared, their sequelae—the fibrous intimal plaques—being the prominent feature.

In spite of these theoretical objections, the results of our experiment suggest strongly that cortisone acetate in the dosage used, or some other ingredient of the cortisone suspension, possesses a distinct fibrolytic activity in the chicken. If true, and our results obviously need confirmation, the possibility of applying this finding to the treatment of human vascular disease must be considered. One point is to be emphasized: There is very little similarity between end-stage coronary sclerosis of chickens and coronary atherosclerosis of man. In the chicken the naturally occurring intimal plaques are essentially fibrous and cellular, and have little or no lipid component as have the human type. There is, therefore, no indication from our experiment that cortisone might be efficacious in the treatment of human coronary artery disease. At the same time there are several human diseases which are accompanied by cellular and fibrous proliferation of the intimal coat of arteries—endarteritis obliterans, rheumatic and syphilitic arteritis, periarteritis nodosa, and diffuse pulmonary vascular sclerosis. Attempts to influence the course of the stenosing arterial lesions in these diseases by cortisone therapy would appear to be justified.

SUMMARY AND CONCLUSIONS

The alleged fibrolytic effect of cortisone has been investigated by determining its effect on the number and the size of naturally occurring fibrous plaques of the coronary arteries of elderly chickens. Under the conditions of the experiment an apparent fibrolytic effect was achieved: A highly significant reduction in the number of arterial lesions was demonstrated in the cortisone-treated birds as compared with the controls, and there was also a reduction in the size of individual lesions.

ADDENDUM

Since this paper was submitted for publication, further evidence has been obtained that activity of the adrenal cortex may be concerned in the progression of "spontaneous" arteriosclerosis in chickens. White leghorn cockerels have been subjected to bouts of stress several times a day for one year, and on comparing the number and the size of fibrous intimal plaques of the coronary arteries in these birds with those in control birds a definite regression of the disease process has been found. The details of this experiment will be reported later.

The statistical analyses reported in this paper were made by Mr. G. B. Oakland, head of the Biometric Unit, Science Service, Department of Agriculture of Canada; technical assistance in the conduct of the experiment was given by Mr. W. J. Conroy, and the serial sections were prepared by Mrs. G. Strickland, Miss M. Long and Mrs. M. Coutts.

HYPERTENSIVE CARDIOVASCULAR DISEASE

Nature and Pathogenesis of the Arteriolar Sclerosis Induced by Bilateral Nephrectomy as Revealed by a Study of Its Tinctorial Characteristics

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AND
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THE ASSOCIATION of arteriolar sclerosis and hypertensive cardiovascular disease is well established and constitutes a fundamental pathological feature of the latter disorder. However, the mechanism whereby this change is induced in the arterioles and the relative role played by the elevation of blood pressure as compared with other factors involved in the genesis of the disease remain unsettled. The demonstration of the possibility of inducing hypertension by nephrectomy [1] with the development of the hemodynamic [2] as well as of the pathological [3] changes characteristic of this disorder in the course of a month permits a better study of the problem, for it enables one to follow the development of the morphological changes in a manner not possible in the human patient, in whom only the end result of the process is available for examination.

The cardiovascular lesions which we have already described [3] are characterized by necrosis and fibrosis of the myocardium and by arteriolar sclerosis. The lesions of the latter, involving the smaller arteries and arterioles, may be divided into three separate categories,[3b] (1) the "hyperacute," (2) the "acute," and (3) the "subacute and chronic" types. The hyperacute lesions are characterized by fulminant necrosis and swelling of the media giving rise to a smudged or smeared appearance. The acute lesion is characterized primarily by an altered appearance of the necrotic

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Dr. Turner is Fellow of the Dazian Foundation for Medical Research.

From the Departments of Experimental Medicine and Pathology, Southwestern Medical School of the University of Texas.

This work was supported by a grant from Mr. and Mrs. Grady Vaughn to the Southwestern Medical Foundation, for the study of hypertension.

1. Grollman, A.; Muirhead, E. E.; and Vanatta, J.: Role of the Kidney in the Pathogenesis of Hypertension as Determined by a Study of the Effects of Bilateral Nephrectomy and Other Experimental Procedures on the Blood Pressure of the Dog, Am. J. Physiol. **157**:21, 1949.

2. Grollman, A.; Turner, L. B.; Levitch, M., and Hill, D.: Hemodynamics of the Bilaterally Nephrectomized Dog Subjected to Intermittent Peritoneal Lavage, Am. J. Physiol. **165**:167, 1951.

3. (*a*) Muirhead, E. E.; Vanatta, J., and Grollman, A.: Tissue Changes in Bilaterally Nephrectomized Dogs, Arch. Path. **48**:234 (Sept.) 1949. (*b*) Muirhead, E. E.; Turner, L. B., and Grollman, A.: Vascular Lesions of Dogs Maintained for Extended Periods Following Bilateral Nephrectomy or Ureteral Ligation, A. M. A. Arch. Path. **51**:575 (June) 1951.

media, the shape of which is retained, the necrotic muscle assuming the compact appearance of early hyalinization. The subacute and chronic changes include a series of alterations involving all three layers of the vessel wall, culminating in total hyalinization, at which stage it is not always possible to identify the contribution made to the lesion by the various layers.

In general, the above-mentioned three types of lesions, which have been described in detail in a previous communication,[3b] are related to the survival time of the dog following bilateral nephrectomy. There is, however, some degree of overlapping of these lesions between various periods of survival. The hyperacute lesions (fulminant arteriolar necrosis) are most notable in animals succumbing within 10 days. The acute lesions occur thereafter but are especially prominent in animals succumbing between 10 and 20 days. The subacute and chronic lesions have been observed mainly in a series of nephrectomized animals living for 23 to 70 days.[2]

It is our opinion that the hyperacute and acute lesions resemble the arteriolar sclerosis observed in human malignant, or rapidly progressive, hypertension.[4] The subacute and chronic lesions resemble the arteriolar sclerosis of patients dying with benign hypertension. The present paper adds additional evidence in support of this view and contributes to an elucidation of the nature and the genesis of the observed lesions.

METHODS

Blocks of tissue from two separate groups of bilaterally nephrectomized dogs were chosen for the study of lesions. The first group consisted of 14 dogs surviving up to 18 days [1]; the second group consisted of seven dogs surviving from 23 to 70 days after removal of the second kidney.[2] A description of both groups as studied with the routine hematoxylin and eosin stain has been reported.[5] For the present study, serial sections, 5μ thick, were prepared and stained with the following nine stains: hematoxylin-eosin, elastic-tissue, reticulum, phosphotungstic acid-hematoxylin, Goldner's trichrome, Van Gieson, periodic acid-Schiff reagent, aniline-blue and toluidine-blue. A total of 1,100 slides were studied. As controls, tissue sections from one of the removed kidneys were prepared in the same manner.

The technics used for the nephrectomy, for the determination of the mean arterial pressure, and for maintaining the nephrectomized dog alive have been described elsewhere.[6]

In all animals included in the present study arterial hypertension developed following the removal of the second kidney.[7] The mean arterial pressure reached hypertensive values, ranging between 150 and 200 mm. Hg, within seven to 14 days and was sustained at these levels until shortly before the death or killing of the animals.

RESULTS

Hyperacute and Acute Lesions.—The early phase of the hyperacute lesion was characterized by hyaline swelling of the sarcoplasm of the smooth muscle fibers of the media, accompanied usually by pyknosis of the nuclei. In some vessels individual

4. Muirhead, E. E., and Grollman, A.: A Case of Acute Renal Insufficiency in Man Manifesting the Arterial Necrosis Observed in Dogs Following Bilateral Nephrectomy, Am. J. Med. **10**:780, 1951.

5. Muirhead, Vanatta, and Grollman.[3a] Muirhead, Turner and Grollman.[3b] Muirhead and Grollman.[4]

6. (*a*) Grollman, A.: Experimental Hypertension in the Dog, Am. J. Physiol. **147**:647, 1946. (*b*) Grollman, A.; Turner, L. B., and McLean, J. A.: Intermittent Peritoneal Lavage in Nephrectomized Dogs and Its Application to the Human Being, A. M. A. Arch. Int. Med. **87**:379 (March) 1951. Grollman and others.[1]

7. Grollman, Muirhead, and Vanatta.[1] Grollman, Turner, and McLean.[6b]

fibers or isolated groups of fibers revealed these changes. The advanced phases showed loss of identity of the individual smooth muscle fibers with smudging of the necrotic remains. The smudged, necrotic media usually spread into the adventitia, leaving the endothelium intact and the luminal blood unclotted. Some degree of swelling of the media toward the lumen caused the latter to become narrowed. Classic thrombosis was not observed. An occasional vessel demonstrated a marked spreading of the necrotic media into the adjoining connective tissue, forming an irregular, geographical mass, near the center of which the intact endothelium could still be identified. These changes were segmental, involving either the entire circumference or a part of the wall, as demonstrated by serial sectioning. When the necrotic media spilled into the lumen the endothelium at times was not identifiable, and the appearance of solid tubes of the necrotic material, thrombonecrosis, was obtained.

The acute lesions manifested variations in the physical characteristics of the necrotic media, which changed from a finely granular structure to a coarsely lumpy and partially hyaline structure to a completely smooth and hyalinized wall. These transitions suggest that total hyalinization of arterioles, including the type associated with a narrowed and eccentric lumen (Fig. 1*D*), resulted from these changes in the necrotic media.

The necrotic media and three other muscular structures (the smooth muscle of the media of intact blood vessels, including those in the control sections of the normal kidneys, the smooth muscle of the muscular coat of the gastrointestinal tract, and the muscle fibers of the myocardium) all showed the same color when stained with a given stain. The colors induced by the various stains were as follows: hematoxylin and eosin, pink; elastic-tissue stain, gray; phosphotungstic acid-hematoxylin, deep blue; trichrome stain, vermilion; Van Gieson, yellow; aniline-blue, red; toluidine-blue, blue. The identity of the tinctorial properties of all these structures indicates that in the hyperacute and acute vascular lesions one most likely is dealing with alterations in the smooth muscle fibers which result in hyalinization. It seems unlikely that the smudged material represents protein, forced into the vascular wall through the endothelial lining, which consistently shows the same staining characteristics as smooth muscle with multiple staining techniques.

The internal elastic lamina, as demonstrated by the elastic-tissue, phosphotungstic acid-hematoxylin, and aniline-blue stains, either became markedly attenuated or was totally destroyed, its remains becoming incorporated in the necrotic media. The narrowed and straightened appearance of the elastica interna reflected the stretching of the necrotic, partly hyalinized media.

The toluidine-blue stain did not reveal metachromasia, which indicates that there was no increase in the amount of ground substance between the muscle fibers, of which there is little normally present in the media. The reticulum stain demonstrated a disruption of the reticulum culminating in either a marked decrease of reticulum or of reticulum fibers or their total disappearance.

The lumpy and granular change of the sarcoplasm of necrotic myocardial fibers was accentuated especially by these various stains. A greater incidence of hyperacute and acute arterial and arteriolar lesions was noted within the area of myocardial necrosis in the present study than previously.[39]

Subacute and Chronic Arteriolar Lesions.—The seven dogs of this group survived between 23 and 70 days (average, 37.5 days) and exhibited acute lesions with

the characteristics described above, as well as subacute and chronic lesions. These lesions were particularly prominent in the gastrointestinal tract, the heart, the adrenals, and the urinary bladder. The subacute and chronic lesions, as previously described,[3b] represented a series of changes, occurring singly or in combination, and involving all layers of the vessel wall. These changes are outlined in Table 1.

1. Hematoxylin and eosin stain (Fig. 1*B*). The results obtained with this stain have been recorded in a separate report.[3b] The present observation conforms in every respect with those previously described.

2. Elastic-tissue stain. With this stain hyalinization of an arteriole or small artery may include (*a*) disappearance of the internal elastic lamina, making im-

TABLE 1.—*An Outline of the Series of Changes in Small Arteries and Arterioles (Arteriolar Sclerosis) Considered as Subacute and Chronic*

I.	Nature of the lesion as observed in:
	A. The intima
	1. Subendothelial hyalinization
	2. Subendothelial fibrosis
	3. Thickening of the internal elastic lamina
	4. Attenuation or destruction of the internal elastic lamina
	B. The media
	1. Hyalinization
	2. "Hyperplasia" (fibrous replacement)
	3. Degeneration: remnants of necrotic muscle, atrophy of muscle
	4. Hypertrophy
	C. The adventitia: concentric fibrosis
	D. All layers: total hyalinization
II.	Degenerative features
	A. Subendothelial hyalinization
	B. Damage of internal elastic lamina
	C. Destruction of internal elastic lamina
	D. Hyalinization of the media
	E. Atrophy of the media
III.	Reparative features
	A. Subendothelial fibrosis
	B. Medial fibrosis
	C. Adventitial fibrosis

possible identification of the layers involved in the hyalinization; (*b*) attenuation or persistence of the internal elastic lamina with hyalinization of the media (Fig. 2*D*); (*c*) a combination of subendothelial and medial hyalinization (Figs. 2*A* and 2*D*); (*d*) thickening of the internal elastica (Fig. 2*C*). In the last-named case, hyaline material accumulates between the endothelium and the internal elastic lamina with hyalinization of the remaining media. The elastica is usually attenuated but can be identified within the thickened vessel, frequently near the midportion (Fig. 2*D*).

This stain also demonstrated (Table 2) that increased cellularity of the vessel wall associated with thickening may result from changes in the intima or the media alone or from changes in both of these layers.

3. Reticulum stain. The reticulum stain demonstrated that hyalinization of the vessel wall was associated with disruption or disappearance of the reticulum pattern

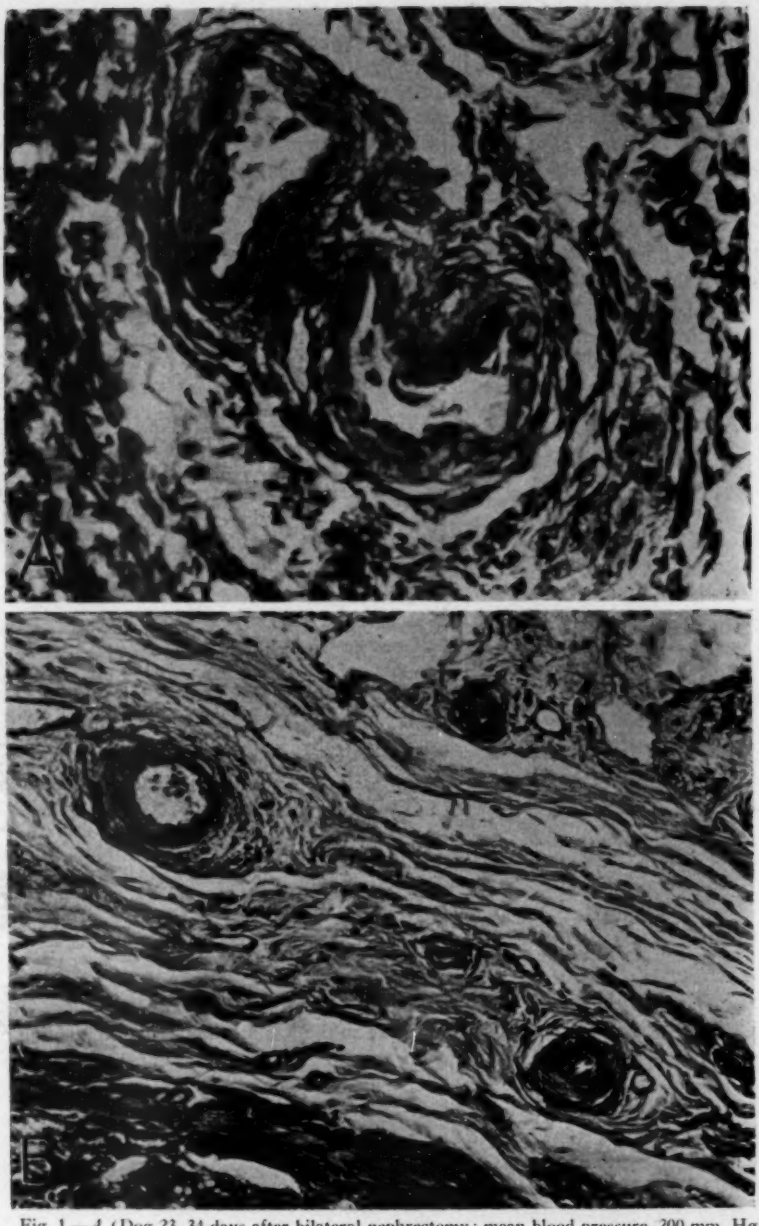

Fig. 1.—*A* (Dog 23, 34 days after bilateral nephrectomy; mean blood pressure, 200 mm. Hg), aniline-blue stain of a small artery in the submucosa of the stomach showing hyalinization beneath the endothelium; × 430. The hyaline material stains similar to smooth muscle and is considered as being derived from the media. This change occurs usually in the subacute and chronic type of lesion.

B (Dog 16, 40 days after bilateral nephrectomy; mean blood pressure, 175 mm. Hg), hematoxylin and eosin stain of arterioles in the submucosa of the large bowel; × 185. These vessels are hyalinized and have a thickened wall and a narrowed lumen. The two smaller arterioles show a smooth hyalinization, whereas the larger one is granular in appearance. Similar lesions of vessels, which are of the subacute and chronic variety, are shown in Fig. 2*D*.

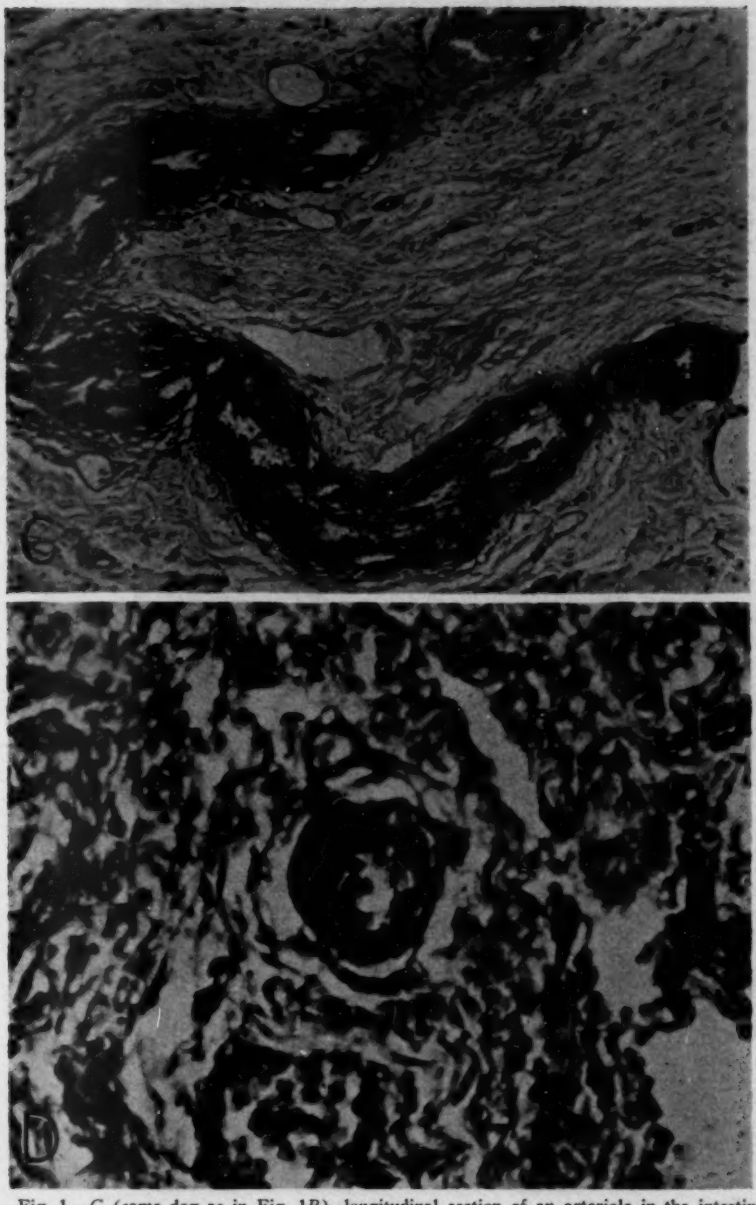

Fig. 1.—*C* (same dog as in Fig. 1*B*), longitudinal section of an arteriole in the intestinal submucosa stained with the periodic acid-Schiff reagent stain; × 150. The lumen is narrowed and the wall is thick and hyalinized. The hyalinized wall partakes of a purplish color.

D (Dog 10, 34 days after bilateral nephrectomy; mean blood pressure, 155 mm. Hg.), arteriole in the gastric submucosa stained with the aniline-blue stain; × 630. The wall is totally hyalinized and thickened, and the lumen is eccentric. The brilliant red stain is similar to that of smooth muscle. This is a subacute and chronic lesion.

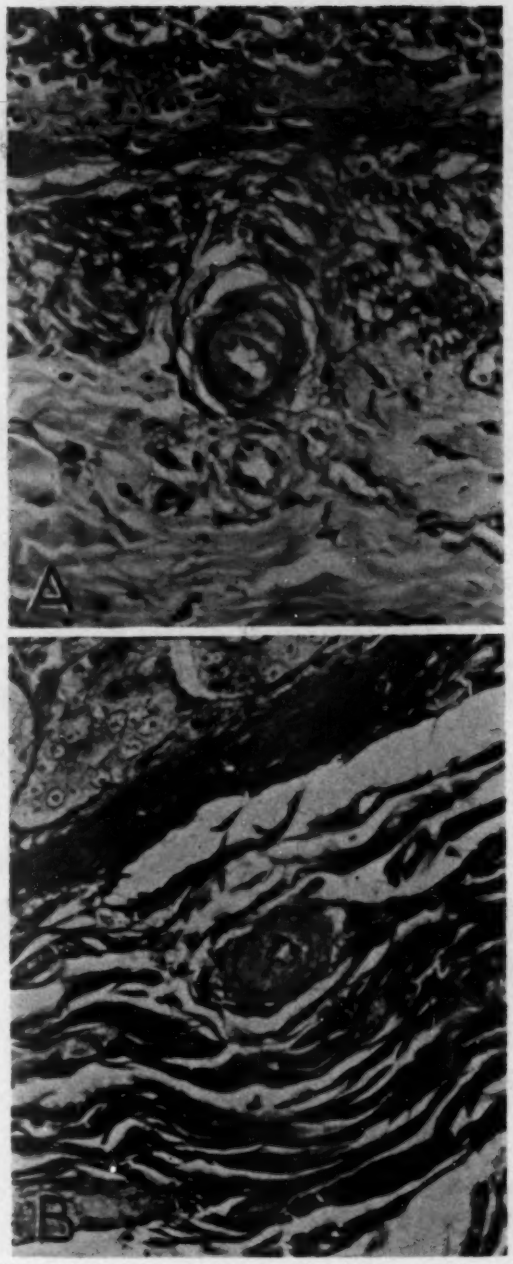

Fig. 2.—*A* (Dog 29, 31 days after bilateral nephrectomy; mean blood pressure, 180 mm. Hg), arteriole of the submucosa of the stomach stained with the elastic tissue stain; × 430. There is an accumulation of a partly hyaline substance between the endothelium and the internal elastic lamina. The lumen is eccentric and narrowed, while the media is atrophic and hyaline. Although the subendothelial material is acellular, it stained like connective tissue. This connective-tissue-like deposit is considered to give rise to a form of subendothelial hyalinization in arteriolar sclerosis.

B (Dog 23, 34 days after bilateral nephrectomy; mean blood pressure, 200 mm. Hg), arteriole in the submucosa of the stomach stained by the aniline-blue method; × 430. It has an eccentrically thickened wall and a narrowed lumen. The wall of this vessel stained light blue, suggesting the presence of connective-tissue-like elements.

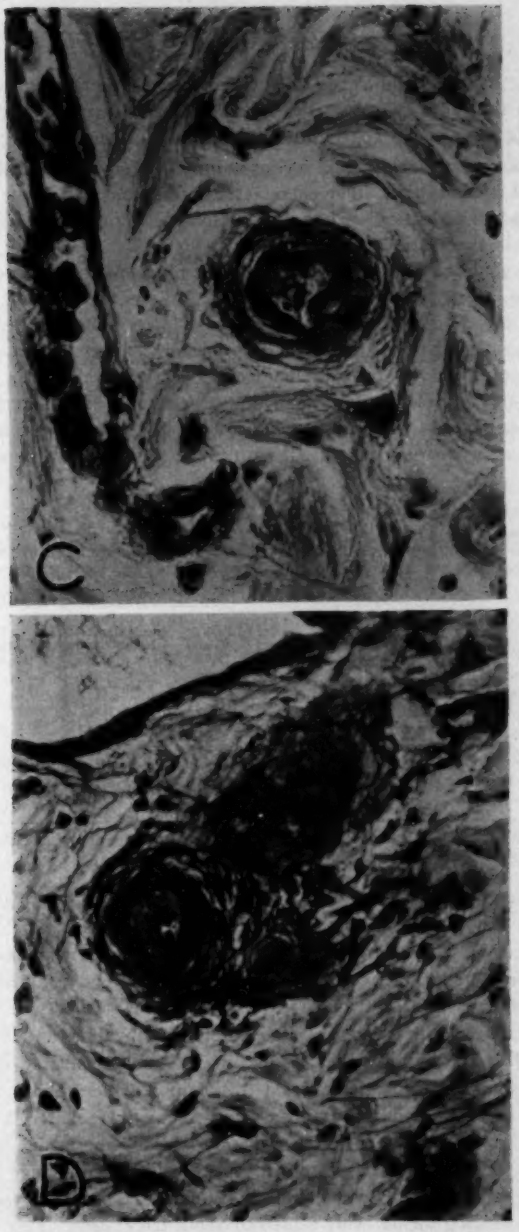

Fig. 2.—*C* (same dog as in 2*B*), arteriole of the submucosa of the intestine stained with the elastic-tissue stain; × 720. The internal elastic lamina is markedly thickened, and the media is partly hyalinized.

D (Dog 16, 40 days after bilateral nephrectomy; mean blood pressure 175 mm. Hg), arteriole in the submucosa of the large bowel stained with the elastic-tissue stain; × 340. The wall is eccentrically thickened and hyalinized, and the lumen is narrowed. The hyalinized areas are shown by the internal elastic lamina. The internal elastic lamina is attenuated but discernible between an accumulation of a hyaline substance between it and the endothelium and the hyalinized media.

of the media. In five of the seven dogs there were disruption and decrease of the reticulum; in the other two dogs the reticulum had entirely disappeared. In two of the animals there was a concentration of reticulum beneath the endothelium.

4. Phosphotungstic acid-hematoxylin stain. This stain revealed two types of subendothelial hyalinization: that characteristic of connective tissue (orange) and another (deep blue) identical with that of the hyalinized necrotic media. The former was observed in three, the latter in two dogs (Table 2). Remnants of the internal elastic lamina could be identified in most vessels revealing a hyalinized media staining deep blue as the intact smooth muscle. Where the subendothelial hyaline material was deep blue, the internal elastica was lost within this material. Certain thickened arterioles showed three other significant changes. Lumpy blue masses, remnants apparently of a necrotic media, were observed within a pale, faintly orange media in two dogs, while in two others the vessels revealed hypercellularity of the media which partook of an orange stain similar to that of the connective tissue. Total hyalinization was associated with an orange rather than a blue stain in some vessels.

TABLE 2.—*Frequency of Lesions as Revealed by Various Stains in a Series of Seven Dogs Surviving Twenty-Three to Seventy Days After Bilateral Nephrectomy**

Lesion	Frequency with Given Stain			
	Elastic-Tissue Stain	Phosphotungstic Acid-Hematoxylin Stain	Trichrome Stain	Aniline Blue Stain
Subendothelial hyalinization	4	5	1	0
Subendothelial fibrosis	2	..	4	3
Thickening of elastica interna	2	1	1	2
Destruction of elastica interna	5	..	..	..
Attenuation of elastica interna	2	..	..	..
Medial hyalinization	4	5	6	6
Medial "hyperplasia" (fibrosis)	1	4	5	5
Total hyalinization	4	5	6	5

* The apparent discrepancies are due in part to the segmental nature of the involvement and in part to the inherent nature of the various stains.

5. Trichrome stain (Goldner). The changes observed in the small arteries and arterioles with this stain were similar to those with the phosphotungstic acid-hematoxylin stain except for coloration. Two types of subendothelial thickening were noted, one stained green, like connective tissue, and the other stained brilliant red, like the smooth muscle of the media (Fig. 1*A*). The former was loose and resembled connective tissue, whereas the latter was hyaline and resembled the hyalinized media. Hyalinized media stained in a manner similar to smooth muscle elsewhere. Most of the animals revealed thickening of the arteries and arterioles as a result of increased cellularity with a green intercellular material. This change was interpreted as a replacement of the layers, especially the media, by connective-tissue elements. Certain vessels did not reveal this advanced change but contained a light-staining media within which lumpy granular red masses, resembling remnants of the hyalinized media, were scattered about irregularly. This was observed in three dogs. In two animals a concentric, whorled collection of green-staining connective tissue was noted around some of the hyaline arterioles, as though a reaction to strengthen the vessels from without were taking place (Table 2).

6. Van Gieson stain. This stain did not lend itself to as detailed a differentiation of the changes as did the other stains. There were only three major changes: (*a*) The entire vessel was hyaline and stained yellow, in which case the contributions of the various layers could not be identified (six dogs); (*b*) the media mainly was involved (six dogs), or (*c*) the vessel was thickened by a combination of a yellowish-staining substance within which pink fibers resembling connective tissue fibers could be identified (four dogs).

7. Periodic acid-Schiff reagent stain (McManus). With this stain, the media of normal blood vessels and the muscularis of the gastrointestinal tract retained a minimal amount of pink coloring. The thickened and altered arteries and arterioles departed from this appearance by assuming a reddish purple color (Fig 1*C*). A similar type of staining was noted in the various alterations. The pink subendothelial hyaline material was noted in three dogs, pink medial hyaline material in three, fibrillar and purplish hypercellular media in three, and red concentric fibers in the adventitia in one dog.

8. Aniline blue stain (Mallory). The changes observed with the aniline blue stain resembled in distribution those observed with the phosphotungstic acid-hematoxylin and trichrome stains. The subendothelial zone was thickened by the collection of material staining blue like connective tissue in three dogs (Fig. 2*B*); thickening of the internal elastic lamina was noted in two dogs. The media when hyaline stained red (Fig. 1*D*) like smooth muscle fibers (six dogs). In two dogs a light-staining media was observed within which were red and lumpy remnants staining in a manner similar to the hyaline media (Table 2). Hypercellular, thickened arteries and arterioles containing a blue fibrillar intercellular material resembling connective tissue were noted in five dogs.

9. Toluidine-blue solution. With the toluidine-blue stain the subendothelial and the medial hyalin stained blue in a similar manner. The former was noted in three dogs; the latter, in all seven of the group. Metachromasia, with a purplish stain, was noted in three different locations. In one dog it was beneath the endothelium. In three dogs it was in the media, and in two dogs it was in the concentric deposit of connective tissue in the adventitia about hyalinized vessels. In all these locations the metachromatic material was fibrillar and resembled fine connective-tissue fibers.

COMMENT

In these studies subendothelial hyalinization and subendothelial fibrosis were not easily differentiated, as in the case of human tissues. The material beneath the endothelium usually stained in a manner similar to connective tissue. An exception appeared in the case of Van Gieson's method, with which this substance was usually stained yellow rather than pink. There was one type of subendothelial hyalinization that differed from the endarteritic type. In this form the staining was similar to that of the hyalinized media, which was considered as indicating that the inner smooth muscle fibers of the media had become hyalinized and swollen and extended toward the endothelium. When this occurred, the internal elastica could not be identified.

Hyalinization of the media was associated with attenuation or destruction of the internal elastic lamina. Rarely, the elastic was thickened. Reduplication of the elastica (elastosis of the intima) was not observed, and this represented a departure from the changes observed in human material.

Fibrosis of the entire vessel wall was demonstrated by thickened vessels staining almost entirely with the connective-tissue stains. Such vessels were at times hypercellular. Hyperplasia of the media in the sense that an increase of smooth muscle fibers had occurred was not observed. In a few instances, hyalinization of the wall (media alone or media and intima) was associated with a concentric thickening of the adventitia. Here the impression was gained that the proliferation represented an attempt to strengthen the vessel wall from without by fibrosis.

These observations made with various differential stains have elucidated certain characteristics of the arteriolar sclerosis developing in dogs subsequent to bilateral nephrectomy and have a bearing, we believe, on the comparable condition occurring under other circumstances. In the hyperacute and acute lesions evidence is presented to support the view that the basic damage is sustained by the smooth muscle of the media. The fact that vessels showing the smudged appearance or the hyaline change have the same staining properties as healthy muscle elsewhere supports the view that the smooth muscle is the structure altered. This view is supported further by the facts that (1) in early hyalinization single fibers are seen to be affected and (2) nuclear changes (pyknosis, karyorrhexis, and karyolysis) occur concomitantly with the tendency toward hyalinization. Ultimately there is a completely hyalinized arteriole with or without a narrowed and eccentric lumen. At times a vessel so altered is stretched rather than narrowed in appearance. Although the endothelium is usually preserved and classic thrombosis has not been observed, the process does not always preserve the endothelium and the lumen. At times the altered smooth muscle spills into the lumen and occluded vessels resembling solid tubes may result.

Our observations indicate the pathogenesis of one form of total hyalinization of small arteries and arterioles. The fundamental injury appears to be sustained by the smooth muscle fibers of the media. In the hyperacute lesion the injury is followed by necrosis and smearing or smudging of the necrotic material, usually toward the adventitia. This material, which is intensely eosinophilic, may be finely vacuolated, finely granular, lumpy-granular, or stringy. This phase resembles the "fibrinoid" necrosis often referred to in the literature. Apparently, in time this altered necrotic sarcoplasm of the smooth muscle changes its physical properties and assumes a more hardened, compact, smooth, or hyaline appearance. Its staining properties, however, remain the same. In its transition toward total hyalinization the hyperacute resembles the acute form of lesion.

In the acute phase the injury to the smooth muscle is less fulminant than in the hyperacute phase, the necrosis of the smooth muscle passing into a hyaline state without a smudged or smeared appearance. The outlines of the muscle fibers are lost and the sarcoplasm merges and forms from the outset a compact structure having a hardened appearance, within which nuclear remnants may persist. Sectioning of the vessel at this stage does not reveal a smooth, totally hyaline structure; instead, the cut surface of this substance frequently appears divided into coarse segments each of which is undergoing hyalinization. It seems apparent that the ultimate change in such a structure is total hyalinization.

In both the hyperacute and the acute form the reticulum network of the media, which bounds the smooth muscle fibers, is either markedly reduced and disorganized or entirely lost. The internal elastic lamina is also affected, being usually destroyed, and its remnants apparently become incorporated in the necrotic media. This is

indicated by the attenuation of the elastica, which remains as a thin line devoid of its normal wavy appearance. At times a segment of the elastica is disrupted and lost in the necrotic media.

Within the myocardium and in the muscularis of the gastrointestinal tract focal areas of necrosis may accompany hyaline arterioles which appear solid or nearly so. It is at such sites that the endothelium appears to be obliterated by the swollen, necrotic media extruded into the lumen. Hemorrhage may occur in the wall of the necrotic vessels, and, with edema, is seen also on occasion within the submucosa of the gastrointestinal tract, the retina, the ciliary body and the choroid coat. Edema occurring alone or in combination with hemorrhage indicates, we believe, an alteration in the capillary structure secondary to the hyperacute and acute arteriolar changes. Larger hemorrhages indicate disruption and leakage of small arteries or arterioles.

The fact that the subacute and chronic arterial and arteriolar lesions, which resemble benign arteriolar sclerosis of the human, are associated with the acute lesions suggests that they all may be pathogenetically related. In the former the various alterations require a period of time for their development and result in the appearance of various transitional forms. Thus, certain thickened vessels containing elements that stain similarly to connective tissue elements also contain remnants of hyalinized smooth muscle of the media. The elastic-tissue stain has revealed that the outer half of a hyalinized vessel is composed of hyalinized media. It has become evident that all types of lesions, through various stages, tend to culminate in total hyalinization.

Thickening of the intima may result from deposition of connective-tissue elements or of a hyaline material between the internal elastic lamina and the endothelium. When this accumulation occurs, there is an attendant atrophy of the smooth muscle of the media, or the media may be attenuated and hyalinized. Thus, even in the presence of the intimal changes, the smooth muscle of the media is also affected. At this stage it is not possible to be certain which change appeared first.

This combination of intimal thickening, either of the hyaline or the proliferative endarteritic type, and atrophy of the media may be considered to result from either of two disturbances: primary intimal thickening with pressure atrophy of the media, or injury of the media with atrophy and resulting intimal thickening of the vessel wall. According to the latter view, the injury, although low grade, by acting over a sufficient period allows a form of repair to occur in the intima. Several collateral observations support the view that the intimal thickening is secondary to medial injury and atrophy. First, in other pathological states, proliferative endarteritis seems to occur whenever there is reason to suspect damage or weakening of the media. Thus it occurs in syphilitic involvement of the media and adventitia or in tuberculosis, near a suppurative process, when the destruction caused by these infections encroaches slowly on nearby vessels; in the lungs when carcinoma spreads along the vessel wall (lymphangitic carcinomatosis); in areas of senile degeneration, following severe inflammation (chronic pyelonephritis), and near infarcts. In all of these examples weakening of the vessel walls seems to occur over a relatively prolonged interval of time. Proliferative endarteritis, according to this view, is a reparative process involving injured vessels. The assumption that the primary lesion involves weakening of the media is also supported by the following observations:

(1) Damage to the media is evident in the acute lesions which occur in association with intimal thickening, and (2) fibrous replacement of the media, a change that may also include the adventitia, accompanies thickening of the intima.

The genesis of arteriolar sclerosis as observed in the present study involves injury of the vessels, which, depending on the degree of severity, gives rise respectively to hyperacute, acute, and subacute or chronic lesions, each of which is characterized by certain specific structural alterations. These various lesions may be present together, or the hyperacute lesion may change to the acute, which in turn may become the subacute or chronic lesion. The ultimate form of the various lesions appears to be total hyalinization of the arterial or arteriolar wall, with or without a narrowed and eccentric lumen. The final stage, designated as arteriolar sclerosis (or arteriolosclerosis), may also involve the smaller arteries.

The specific nature of the factors determining the severity of the lesions is not known. That the observed elevation of blood pressure alone is not the main factor in the development of these lesions is demonstrated by the hyperacute (necrotic) and acute (early hyaline) lesions that appear in the absence of such elevation. Conversely, Goldblatt [8] has observed the absence of these lesions when hypertension has been present for as long as six years. The elevation of blood pressure must be considered, however, as playing an aggravating role in the genesis of the lesion.

The lesions designated as subacute and chronic have both degenerative and reparative features. Subendothelial hyalinization, particularly that resembling hyalinization of the media, damage of the internal elastic lamina, and hyalinization and/or atrophy of the media are considered as degenerative changes; fibrosis of the intima, the media, and the adventitia, either singly or combined, as reparative changes. These observations indicate that the arteriolar sclerosis represents a combination of damage plus the reparative reaction to the damage. It is apparent that the latter makes itself evident in different structures of the body in various modified forms. In small arteries and arterioles the combination of degeneration and repair makes for the lesion designated as arteriolar sclerosis.

It appears, therefore, that the arteriolar sclerosis associated with bilateral nephrectomy in its ultimate phase (total hyalinization) is mediated through an alteration of a necrotic media or through a combination of degeneration and repair of the vessel wall. This considers the various types of lesions as related pathogenetically and differing primarily in the severity of the basic damage.

SUMMARY

The tinctorial properties of the arteriolar sclerosis encountered in the dog following bilateral nephrectomy have been studied by staining nearby segments of the same vessel with nine stains. The observations indicated a relationship between the formerly described hyperacute or fulminant vascular lesions and the subsequently observed acute and subacute-chronic lesions. All three lesions culminate in total hyalinization of the vessel wall, the final phase of arteriolar sclerosis. The basic injury in arteriolar sclerosis involves the smooth muscle of the media of small arteries and arterioles, the variation in the intensity of this original vascular damage

8. Goldblatt, H.: Anatomical Considerations of Hypertension, in Conference on Factors Regulating Blood Pressure, Transactions of the Fourth Conference, 1950, New York, Josiah Macy, Jr., Foundation, 1951, p. 11.

establishing the nature of the morphologic change, that is, the type of lesion. Hyalinization results from two separate changes: (1) necrosis of the smooth muscle followed by hyalinization of the remaining necrotic sarcoplasm, and (2) replacement of the vessel wall by hyalinized connective tissue-like elements.

The morphologic changes in small arteries and arterioles (arteriolar necrosis and arteriolar sclerosis) which we have described are similar to the changes noted in the human patient during the course of various forms of hypertensive cardiovascular disease ("essential hypertension").

The photomicrographs were made by Mr. Robert Sage, Department of Medical Art and Visual Education, Southwestern Medical School of the University of Texas.

Case Reports

PARAPHYSIAL CYST OF THE THIRD VENTRICLE

Report of a Case with Autopsy Observations

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PARAPHYSIAL cysts are rare tumors of the brain, only 65 having been recorded up to the year 1940 (Zeitlin and Lichtenstein [1]). These tumors seldom occur in patients under 20. Eighty-eight per cent of them, according to Stookey,[2] occur between the ages of 20 and 50. In his cases, 72% of the patients were males and 28% were females. If the cyst is diagnosed in proper time, excellent results can be obtained by surgical treatment. If undiagnosed and untreated, the tumor can and usually does eventuate in the sudden death of the patient.

The purpose of this paper is to present a case of paraphysial cyst of the third ventricle and to focus attention on those factors which would aid in diagnosing this condition, so that treatment could be instituted at the earliest opportune time.

CLINICAL OBSERVATIONS

An 11-year-old boy was admitted to the hospital in an unconscious state. At the time of admission there was dilatation of the left pupil; the right pupil was normal in size. Neither pupil reacted to light. The neck was supple. There was some rigidity of the left upper extremity. There was absence of Kernig's sign. Babinski's reflex was present bilaterally, and Hoffmann's reflex, on the left side. In the hospital, the child had a convulsion associated with twitching of the right arm. Respiration and pulse were satisfactory at this time, but within a short while the child became cyanotic. Artificial respiration and oxygen were given to no avail, and the child died. A spinal tap prior to death revealed the fluid to be under no increased pressure. No manometric measurements were taken. The spinal-fluid sugar was 145 mg. per 100 cc. There were 600 red blood cells per cubic millimeter. No white blood cells were noted. The blood sugar was 260 mg. per 100 cc. The urine was not abnormal except for presence of sugar (4+).

For about two months prior to entering the hospital the child had been retiring earlier than usual because of tiredness. Five days prior to entrance, on July 7, he appeared to be quite listless and complained to his mother of a severe generalized headache that seemed to be worse when he assumed an upright position. On his reclining the headache disappeared or was alleviated to some extent. On the day prior to his death he complained of anorexia, nausea and vomiting. On the morning he was admitted to the hospital, an enema was prescribed for apparent constipation. While receiving the enema at home, the child had a convulsion and became unconscious, and was then admitted to the hospital, where he died a few hours after admission.

From First Army Area Medical Laboratory, 90 Church St., New York.

1. Zeitlin, H., and Lichtenstein, B.: Paraphysial Cysts of the Third Ventricle, J. Nerv. & Ment. Dis. **91**:704, 1940.

2. Stookey, B.: Intermittent Obstruction of the Foramen of Monro, by Neuroepithelial Cysts of the Third Ventricle, Bull. Neurol. Inst., New York **3**:446, 1934.

PATHOLOGIC OBSERVATIONS

Thymus.—One of the important findings at autopsy was an enlarged thymus gland, well-encapsulated and weighing 100 gm. The right lobe of the gland measured 6 by 6 by 6 cm.; the left lobe, 7 by 4 by 3 cm. On cut section no remarkable changes were noted. Microscopic examination of the gland revealed the structure to be relatively normal. There was hyperplasia of both the cortical and the medullary portion of the gland. There was an increase in the relative number

Fig. 1.—Dorsal view of the brain, with the corpus callosum retracted laterally, showing the paraphysial cyst suspended from the roof of the third ventricle.

of Hassall's corpuscles, the major portions of which were in various stages of calcification. The cortical portion of the gland seemed to be divided up into rather large collections of thymocytes.

Brain.—After the scalp was reflected in the usual manner, the calvarium was removed. The calvarium measured 3 mm. in thickness except in the midline just behind the area of the bregma. At this point there were two round, smooth, eroded areas of the parietal bone. At one point the bone was of tissue-paper thinness. The

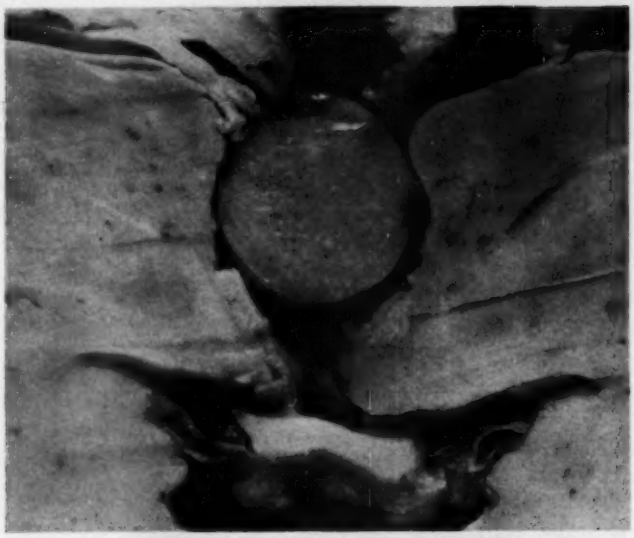

Fig. 2.—Coronal section of the brain and cyst showing the intimate relationship of the cyst and surrounding vital structures.

Fig. 3.—Photomicrograph of the entire cyst. Hematoxylin and eosin; low power magnification.

areas of erosion were on either side of the midline and measured approximately 2.5 cm. at their widest portion. The changes were contiguous with moderately elevated areas of the dura. The raised areas of the dura were due to a combination of two factors: cerebral edema and dilatation of the superior sagittal sinus and the parietal venous lacunae. On reflecting the dura, one noted that the brain was quite edematous. It weighed 1,750 gm. The gyri were flattened and the sulci were narrowed. There was moderate dilatation of the blood vessels over the surface of the brain. When the brain was removed, there was a gush of fluid from the sub-

Fig. 4.—Photomicrograph of the cyst wall, showing the epithelial covering. No tubules are noted. Hematoxylin and eosin; high power magnification.

arachnoid cistern of the pons, which had been nicked. The fluid was under obvious tension. On opening the lateral ventricles by an incision through the cerebral hemispheres, one noted a large amount of intraventricular fluid. The ventricles were dilated. After incision of the corpus callosum, a gray-tan, cystic structure, measuring approximately 1.4 cm. in diameter, was found hanging from the roof of the third ventricle, at a point about 2 cm. from the genu of the corpus callosum (Fig. 1). This round mass seemed to be pressing on the anterior portion of the basal ganglions on the right side. On cut section, after fixation in formaldehyde solution, the mass

was composed of a homogeneous white tissue of a spongy appearance. The tissue had the consistency of solid gelatin. A thin, smooth capsule surrounded the entire mass (Fig. 2). There was a moderate amount of fluid in the third ventricle. No hemorrhage was seen.

Microscopic examination of the cyst (Figs. 3 and 4) disclosed the following features. Focal areas of the surface of the cyst wall were adherent to a normal-appearing choroid plexus. The cyst wall was comprised of dense, flattened, avascular connective tissue, which varied only slightly in thickness. On the inner surface

Fig. 5.—Photomicrograph of the degenerating epithelial cells, which are arranged in a configuration resembling the "sulfur bodies" of actinomycosis. Hematoxylin and eosin; high power magnification.

of the cyst wall there was a flattened cuboidal-columnar layer of epithelial cells. Compression of the cells distorted their shape and accounted for variation in size. The cells had round-to-oval nuclei and contained a fine chromatin network. The cell cytoplasm stained lightly with eosin. Many of the lining cells contained large, clear vacuoles, suggesting that the cells were actively secreting mucus. Definite tubular spaces as described by Zeitlin and Lichenstein [1] were not noted within the cyst wall.

Inside the epithelial layers was a reticular network filled with a basophilic granular material. Large aggregates of the degenerating cellular material formed irregular masses (Fig. 5). Polymorphonuclear neutrophils were present around these granular masses. The degenerating cellular material within the cyst closely resembled the "sulfur granules" of actinomycosis. Bollinger's [3] description of a case of actinomycosis of the brain published in 1887 was based on the appearance of such bodies. Dandy [4] questioned that diagnosis inasmuch as such bodies are characteristic of those noted in the colloid cysts of the third ventricle. In our case numerous stains were consistently negative for the presence of fungi. The cystic contents had a diffuse watery colloid appearance in which were noted desquamated epithelial cells with a slightly foamy cytoplasm, pyknotic, naked nuclei, and red blood cells. Sections taken through the cerebral white cortex demonstrated a thin layer of demyelination.

COMMENT

Paraphysial cysts of the third ventricle of the brain, also referred to as colloid, ependymal, choroid, or neuroepithelial cysts, arise from the velum transversum in the most anterior part of the third ventricle from an embryonic anlage of the paraphysis (Zeitlin and Lichtenstein [5]; Sjövall [6]). The paraphysis is found most fully developed in the lower vertebrates as a paraphysial gland. In the human embryo the paraphysis is only transitorily present. The paraphysial cyst is always situated in the anterior extremity of the third ventricle, between the interventricular foramens and is attached to the roof of the third ventricle by a short pedicle.

In close proximity to the cyst are a number of vital structures which when encroached on produce the symptom complex of this tumor [7] (Fig. 6):

1. Anteriorly is the foramen of Monro. With enlargement, the cyst becomes impacted in the foramen of Monro and interferes with the flow of cerebrospinal fluid. Sudden obstruction of the foramen of Monro may terminate in sudden death. As a rule, the obstruction is intermittent in character, resulting in secondary hydrocephalus of the lateral and third ventricles of the brain. This may cause headache, nausea, vomiting, epileptiform seizures, and sudden episodes of unconsciousness.

2. Posteriorly is the roof of the tela choroidea which transmits the vein of Galen. The acute paroxysms of headache and the severity of them, according to Stookey,[2] are due not only to the sudden obstruction of the foramen of Monro but also to obstruction of the vein of Galen at its point of origin. The vein of Galen is the only venous channel for the drainage of the entire choroid plexus, not only of the

3. Bollinger, O.: Über primäre Actinomykose des Gehirns beim Menschen, München. med. Wchnschr. **35**:789, 1887.

4. Dandy, W. E.: Benign Tumors in the Third Ventricle of the Brain, Diagnosis and Treatment, Springfield, Ill., Charles C Thomas, Publisher, 1934.

5. Zeitlin, H., and Lichtenstein, B.: Cystic Tumor of the Third Ventricle Containing Colloid Material, Arch. Neurol. & Psychiat. **39**:268 (Aug.) 1937.

6. Sjövall, E.: Ueber eine Ependymcyste embryonalen Charakters (Paraphyse?) im written Hirnventrikel mit tödlichem Ausgang, Beitr. path. Anat. **47**:248, 1910.

7. Davidoff, L. M., and Dyke, C. M.: Congenital Tumors in the Rostral Portion of the Third Ventricle, Bull. Neurol. Inst. New York **4**:221, 1935.

third but of the lateral ventricles. There is no collateral circulation of the choroid plexus. This results in a concomitant sudden interference with the venous outflow of the third ventricle.

3. Inferiorly are the floor of the third ventricle and the optic chiasm which lies just beneath it. Encroachment on the floor of the third ventricle involves not only the optic chiasm but also the hypothalamic nuclei. Pressure on the optic chiasm disturbs the blood flow to the optic nerves causing visual disturbances. The outstanding manifestation of pressure on the hypothalmic nuclei is hypersomnia. This differs from the somnolence and stupor which develop in the course of intracranial pressure in that the patient notices a compelling desire to sleep during his hours of active work. The patient can be aroused easily and answers questions. In protracted cases, the uncontrollable drowsiness becomes more overwhelming and is usually associated with such hypothalamic symptoms as disturbances of body

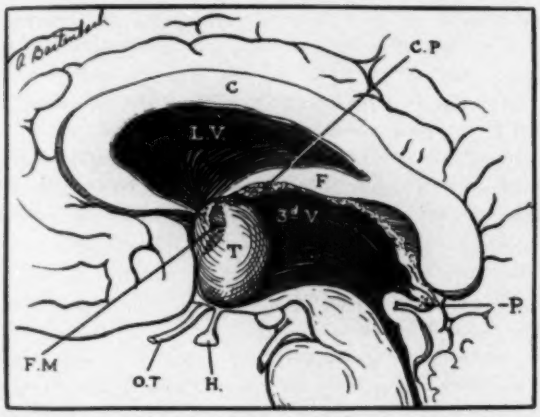

Fig. 6.—Schematic drawing from Zeitlin and Lichtenstein,[1] showing a paraphysial cystic tumor (*T*) suspended from the anterior roof of the third ventricle, together with the neighboring structures: the foramen of Monro (*F. M.*), the opening between the third and the lateral ventricles, obstructed by the tumor; the optic nerve (*O. T.*); the hypophysis (*H*); the corpus callosum (*C*); the fornix (*F*); the pineal body (*P*); the choroid plexus (*C. P.*); a lateral ventricle (*L. V.*); the dilated third ventricle (*3d V.*). This illustration (Fig. 1 of Zeitlin and Lichtenstein [1]) is reprinted with the permission of the *Journal of Nervous and Mental Diseases.*

temperature and disorders of personality (Zeitlin and Lichtenstein [1]). Diabetes insipidus also results from the excessive pressure on the floor of the third ventricle.

4. Laterally are the medial nuclei of the thalamus which form the lateral wall of the third ventricle. Pressure in this area results in mental disturbances and disorders of temperature regulation.

From the standpoint of symptomatology the manifestations of the paraphysial cyst can be divided into early and late stages. In the early stages the tumor produces practically no symptoms, but as it enlarges and encroaches on surrounding structures the various symptoms become more pronounced. Early the patient suffers sudden severe intermittent headaches, relieved by changes in position of the head. This is the most characteristic symptom of the paraphysial cyst. It is followed by vomiting, hypersomnolence, disturbances of vision, and epileptiform

seizures. Late changes consist of sudden losses of consciousness, mental disturbances, diabetes insipidus, hyperthermia, paresthesias, and weakness of the legs. Because of the protean nature of the symptoms occurring in this disease entity, the diagnosis of the true condition, as a rule is not made.

Ventriculography is a valuable adjunct in making the diagnosis, inasmuch as the x-ray picture is almost pathognomonic for this condition. On radiologic examination, the posterior portion of the third ventricle is visualized readily, while the anterior portion directly below and in front of the foramen of Monro is obscured by a filling defect. Of all the tumors of the brain, the paraphysial cyst offers the best prognosis because of its position and its accessibility for successful removal if diagnosed correctly.

SUMMARY

A case is presented of a paraphysial cyst of the third ventricle in a 11-yr.-old child.

Severe intermittent headaches relieved by positional changes of the head is the diagnostic sign leading most frequently to the detection of the tumor. Ventriculography shows a pathognomonic picture: The posterior portion of the third ventricle is visualized, while directly below and in front of the foramen of Monro, the ventricle is obscured by a filling defect.

If the tumor is diagnosed correctly and removed, the patient has an excellent prognosis.

The photographs of the gross specimen were furnished by the Armed Forces Institute of Pathology, Washington 25, D. C.; the photomicrographs were taken by Mr. David Skelton, Meadowbrook Hospital, East Meadow, Long Island.

Figure 6 (Fig. 1 of Zeitlin and Lichtenstein [1]) is reprinted with the permission of the *Journal of Nervous and Mental Diseases.*

STREPTOCOCCUS HEPATITIS

PAUL KOTIN, M.D.
AND
E. M. BUTT, M.D.
LOS ANGELES

PATHOLOGIC changes in the liver associated with local infection are rare. When seen, they are usually in association with chronic, not acute, infectious processes, such as tuberculosis or syphilis. This is in marked contrast with lesions seen in toxic states, in which liver findings are frequent. The observation of beta hemolytic streptococci diffusely distributed throughout the liver at autopsy has been unreported since MacMahon and Mallory[1] comprehensively reviewed the findings in 1931.

In the case reported here, with autopsy observations, lesions were demonstrated in which beta hemolytic streptococci were found diffusely distributed throughout the liver. The pathologic findings in the liver were those of acute focal hepatic necrosis.

REPORT OF A CASE

E. H., a 56-year-old white man, was admitted to the Los Angeles County Hospital on Feb. 5, 1950, at 8 p. m. He complained of hoarseness, dyspnea, edema, and loss of weight of six months' duration. In the week prior to entry the signs and symptoms of cardiac decompensation had become much aggravated. The patient further noticed at this time rapid onset of jaundice, associated with frank asthenia, malaise, chilliness, and prostration. The past history was essentially noncontributory.

When physically examined, the patient was acutely ill, emaciated, and icteric. The pulse rate was 74, and the temperature was 97.4 F. The respirations were 22 per minute, and the blood pressure was 110/60. The scleras were icteric. The pupils reacted to light and accommodation. No adenopathy or masses were noted in the neck. In the lungs, moist rales were heard bilaterally at the bases. No dullness or adventitious sounds were elicited. The cardiac borders, rhythm, and rate were all within normal limits. The tones were distant, and no murmurs were heard. There was diffuse moderate abdominal distention. The liver was palpable 3 finger-breadths below the right costal margin and was moderately tender. The spleen was not palpable or tender. The kidneys were neither palpable nor tender. No rigidity was elicited. No abnormal rectal findings were obtained. The extremities revealed pitting edema to the level of the mid-thighs. The reflexes were physiologic.

Laboratory studies showed a hemoglobin content of 14 gm. per 100 cc.; a white blood cell count of 14,400, with a differential count of 84% of polymorphonuclear granulocytes and 16% lymphocytes. The blood culture was negative. The icterus index (serum) was 72 units. The albumin-globulin ratio was 2.8/3.9. The carbon dioxide-combining power (plasma) was 32 volumes per cent, and the blood nonprotein nitrogen was 77. The blood chlorides amounted to 608 mg. per 100 cc. of plasma or serum.

Although acutely ill, the patient remained afebrile, and in spite of emergency therapy, died 22 hours after admission. The autopsy was performed four hours post mortem.

From the Departments of Pathology of Los Angeles County Hospital and the University of Southern California School of Medicine.

1. MacMahon, H. E., and Mallory, F. B.: Streptococcus Hepatitis, Am. J. Path. **7**:299-325, 1931.

AUTOPSY

Gross Observations.—The central nervous system was not examined. The neck was essentially normal.

The heart weighed 390 gm. There was moderate dilatation of the left ventricle and atrium. The myocardium of the left ventricular wall measured 15 to 17 mm. There was minimal hypertrophy of the trabeculae carneae and papillary muscles. The lungs were emphysematous and showed bilateral hypostatic congestion.

The abdomen when opened revealed 350 cc. of straw-colored fluid (specific gravity, 1.012) free in the peritoneal cavity. The liver was at the costal margin. Omentum was adherent to the peritoneal surface of a McBurney surgical incision. The stomach was dilated and contained 300 cc. of partially digested food. The

Fig. 1.—Masses of streptococci in an endothelial cell; × 1,000.

mucosa was hyperemic and presented an ulcer 1 cm. in diameter on the lesser curvature, 3 cm. proximal to the pyloric ring. The remainder of the gastrointestinal tract was unremarkable. The pancreas was uninvolved. The lower border of the liver was 2 cm. below the right costal margin in the midclavicular line. The capsule was wrinkled, and the surface was coarsely granular. The color was pale yellow. The surface cut with increased resistance and was pale yellow-tan. The lobular structure was distorted, with loss of normal vascular relationships. The venous channels were engorged. The consistency of the organ was diffusely firm. However, grossly visible yellow-orange deposits of amorphous material dotted the cut surface. In areas where these coalesced, the liver was softened. The gallbladder and ducts showed no abnormalities. The spleen weighed 300 gm. Its capsule was wrinkled, and its surface was reddish purple. On section, the pulp was engorged, and the trabeculae were thickened. The kidneys weighed 370 gm. together. Their cap-

sules stripped easily and left a finely granular surface. The cut surface showed no changes. The remainder of the genitourinary tract was free of variations. The aorta showed moderate atherosclerosis of the abdominal portion.

Microscopic Observations.—The chest organs presented nonspecific findings. The abdominal viscera showed passive-congestion changes. The liver sections stained with hematoxylin and eosin showed signs of congestive cirrhosis, with a striking increase in the connective tissue deposited centrally. Focal areas of necrosis were irregularly distributed throughout sections taken from all lobes of the liver. The necrosis tended to be zonal in some areas in that it was chiefly adjacent to the connective tissue of the liver. The necrotic cells were clumped in

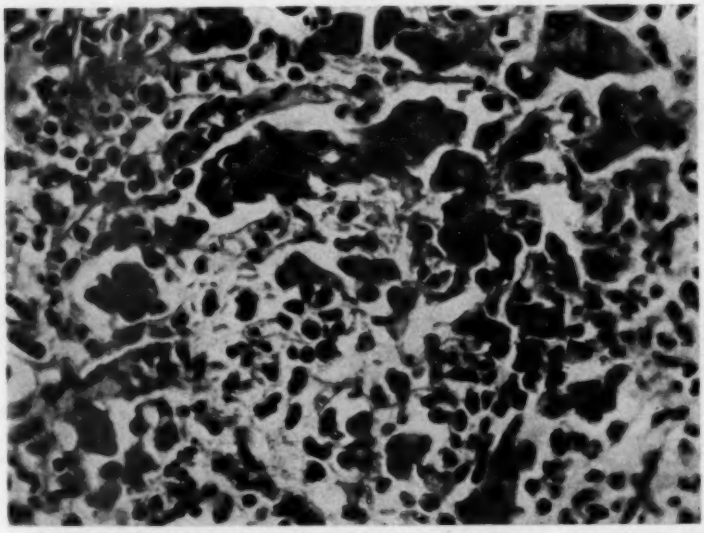

Fig. 2.—Acute inflammatory-cell infiltration with focal necrosis; × 260.

isolated areas. Many of the liver cells showed fatty infiltration, with vacuole formation. The cytoplasm of the liver cells was eosinophilic and granular. Macrophages were present in considerable numbers, and many were swollen with ingested necrotic debris. MacMahon and Mallory [1] reported that phagocytosis was limited solely to the macrophages. The sections studied confirmed this observation. Central veins were intensely engorged and distended. A few contained thrombi of fibrin and formed blood elements. There was a diffuse inflammatory-cell infiltration consisting equally of lymphocytes, polymorphonuclear granulocytes, and histiocytes with numerous eosinophils and an occasional plasma cell. The cellular infiltration was most acute in the sections adjacent to the necrotic areas. Gram-stained sections of liver from all lobes showed numerous clumps of Gram-positive cocci in diplococcoid and circular chain forms. Gram-stained sections from the remaining visceral organs showed no bacteria.

Anatomical Diagnosis.—Acute hepatitis due to infection (beta hemolytic streptococcus); acute focal hepatic necrosis due to infection (beta hemolytic streptococcus); peptic ulcer of the stomach; senile arteriosclerosis; benign nephrosclerosis.

Bacteriologic Studies.—A postmortem blood culture was sterile. Impression smears and swab smears from the liver showed moderate numbers of Gram-positive cocci in diplococcoid and chain forms. Cultures taken through seared surfaces and from washed ground-liver tissue yielded beta hemolytic streptococci of Lancefield's A group. Parallel cultures from all other organs were sterile except for Proteus vulgaris, which grew from the kidney.

A saline suspension of the cultured organisms was injected intravenously into two rabbits. One died after 3 days; the other, after 8 days. Both had positive blood cultures immediately post mortem. The liver of one showed six subcapsular miliary abscesses, measuring 1 to 3 mm. in diameter, from which Lancefield A beta hemolytic streptococci were obtained. Cultures of the spleen and the lung of this rabbit also grew streptococci of the same type and group.

COMMENT

A review of the literature and of 7,500 autopsy records of the Los Angeles County Hospital revealed that the condition described is rare. This rarity is unusual when one considers that disease produced by this group of organisms is usually associated with invasion of the blood stream. The liver and the spleen with their sinusoidal type of anatomy should be especially prone to such findings. Swift [2] stated:

> Streptococci may enter the blood stream in one of three ways: (1) directly through a vein, a rare occurrence in the case of the hemolytic streptococci, but probably fairly common with nonhemolytic varieties; (2) from a thrombophlebitis, contiguous to focally infected tissues, where thrombi originating in the diseased veins and containing streptococci break off and pass into the general circulation; (3) from infected lymphatic vessels, a much rarer occurrence than is the second contingency.

These organisms, in the form of emboli, usually localize in the lung, with resultant infarction. If the emboli are small, they may pass through the lung into the systemic circulation. These, as do infected emboli from the leaflets of the mitral and aortic valves, cause multiple embolization with infarction and hemorrhage. The mode of action of the emboli is by mechanical means as well as by a specific local toxic effect of the streptococci. The resultant lesions, at autopsy, show pathologic alterations on both bases. The local hepatic lesions reported here may be part of this mechanism.

Hepatic localization of bacteria is an unusual finding. The liver characteristically is highly resistant to infection. Extensive intra-abdominal infectious processes may exist for long periods without any demonstrable hepatic lesions. This resistance is not absolute immunity, as evidenced by multiple liver abscesses following pylephlebitis of the portal vein and by the hepatitis reported here.

The lesion of acute focal hepatitis seen here was first described and named by Lande [3] as an "acute focal necrotizing hepatitis." This, according to MacMahon

2. Swift, H. F.: The Streptococci, in Dubos, R. J.: Bacterial and Mycotic Infections of Man, Philadelphia, J. B. Lippincott Company, 1948.

3. Lande, K.: Ueber akuteherdformige nekrotisierende Hepatitis, Frankfurt. Ztschr. Path. **34**:221, 1926.

and Mallory,[1] is in some instances the result of actual presence of bacteria in the liver. Five cases, three of acute and two of chronic lesions with healing, were reported by these authors. The livers in which the process was acute showed necrosis of the focal type. The two with healed lesions showed isolated areas of cirrhosis and the patients had histories and findings suggestive of antecedent bacterial hepatitis. In the cases in which histories were complete, a definite antecedent or associated bacteremia or septicemia was noted. No attempt was made in their report to explain the localization of the bacteria in the liver. They noted instances of focal necrosis of the liver similar to that seen in the reported cases where no streptococci were evident. They stated that the lack of a demonstration of organisms does not exclude streptococci as the cause. MacMahon [4] noted that streptococci, acting locally, can produce considerable change and in a very short time be killed and completely removed. When such bacterial foci are small, complete healing may occur. When they are large, abscess formation and isolated patches of cirrhosis usually result.

SUMMARY

An instance of hepatitis caused by beta hemolytic streptococci is reported. The localization of bacteria may have been facilitated by the hepatic circulatory stasis with pooling of blood following the prolonged decompensation and cirrhotic changes in the liver. The poor nutritional state of the patient is an additional factor that combined to diminish local tissue resistance to the point where acute focal hepatic necrosis followed streptococcal localization.

4. MacMahon, H. E.: Streptococcus Hepatitis, Am. J. Path. **7**:578, 1931.

Laboratory Methods and Technical Notes

A BENZIDINE-THIONINE METHOD FOR THE DEMONSTRATION OF HEMOGLOBIN IN FORMALDEHYDE-FIXED, PARAFFIN-EMBEDDED TISSUE

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NEW HAVEN, CONN.

In order to demonstrate hemoglobin in smears or sections, many methods employ the benzidine reaction. Benzidine is oxidized by hydrogen peroxide (which is decomposed) in the presence of the heme group, thereby staining sites containing hemoglobin brown or black. If the hemoglobin is confined to vessels, it is easily identified, but when it is scattered throughout the section in small amounts, these colors are easily confused with pigments of a similar hue, such as iron deposits and formaldehyde pigment, if such pigments are present in these tissues.

The exact reaction between benzidine and hemoglobin is obscure. According to Hawk and Bergeim,[1] the hemoglobin catalyzes the decomposition of hydrogen peroxide, and the liberated oxygen oxidizes the benzidine. An excess of peroxide interferes with the reaction by destroying the catalytic power of the blood and by reacting with the benzidine itself, with the formation of products which appear to have an inhibitory action.

Lillie [2] pointed out that certain basic dyes such as toluidine blue O or thionine may stain erythrocytes brilliant green or yellowish green. The following method for formaldehyde-fixed, paraffin-embedded tissue utilizes a combination of benzidine and thionine, producing with hemoglobin a well differentiated olive green color, which is not easily confused with other pigments present in the same section.

Tissue fixed in buffered formaldehyde solution (pH 7)[2] or alcohol-formaldehyde solution has given the best results; alcohol has given only fair results, and dichromate fixatives, poor or no staining at all.

Most benzidine preparations fade rather rapidly on exposure to heat or light, but sections stained by this procedure have shown no appreciable fading after more than a year.

Benzidine reagent $(C_6H_4)_2(NH_2)_2$ or benzidine base should be used; benzidine dihydrochloride $(C_6H_4)_2(NH_2)_2 .2HCl$ is not suitable.

METHOD

1. Fix tissue in formaldehyde solution U. S. P. (40 per cent) diluted 1:10.
2. Embed tissue in paraffin in the usual manner.
3. Cut sections at 6 microns.
4. Hydrate sections down to water.
5. Prepare a stock staining solution as follows:

Solution A.	Thionine	0.25 Gm.
	Distilled water	100 cc.
Solution B.	Benzidine	1 Gm.
	Absolute methyl alcohol	100 cc.

Dissolve separately. For use combine equal parts of solution A with B and filter. The combined solutions last for about two weeks.

Stain in the above solution for five minutes, and carry slides individually through the following steps.

From the Department of Pathology, Yale University School of Medicine.

1. Hawk, P. B., and Bergeim, O.: Practical Physiological Chemistry, ed. 10, Philadelphia, The Blakiston Company, 1931.

2. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.

6. Place in a 10 per cent solution of hydrogen peroxide in absolute ethyl alcohol for one minute and agitate gently. This solution should be freshly prepared.

7. Differentiate with microscopic control in the following solution:

Absolute ethyl alcohol	100 cc.
Glacial acetic acid	2 drops

8. Rinse quickly in absolute alcohol; this continues differentiation.

9. Clear in two changes of toluene.

10. Mount in permount.[3]

RESULTS

Hemoglobin is stained olive green or greenish brown whether present in red blood cells or, for example, in casts in tubules of the kidney. Hemosiderin is changed from yellow to brown. The color of hematoidin crystals and bile pigments, either bilirubin or biliverdin, is unaltered; iron in calcified vessels stains blue.

3. Permount® is made by Eimer & Amend, New York.

Books Received

MEDICAL NEUROPATHOLOGY. By I. Mark Scheinker, M.D., assistant professor of neuropathology and assistant professor of neurology, University of Cincinnati College of Medicine; attending neurologist, Cincinnati General Hospital, Cincinnati; consultant neurologist, Veterans Administration Hospital, Fort Thomas, Ky.; consultant neuropathologist, United States Public Health Service, Lexington, Ky. Pp. 385, with 186 illustrations. Price $10. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1951.

It is indeed refreshing to encounter a new approach to a time-honored subject. In the past few years there have appeared numerous texts of neuropathology, all of which follow a more or less uniform mode of presentation. The texts differ widely, of course, in merit, but they follow a traditional pattern. Here is a volume which tries to diverge from tradition. The attempt is made to integrate neuropathology with general pathology and with internal medicine. The whole field of pathology of the nervous system is not covered in this volume, but only those parts which are relevant to internal medicine.

The brain manifestations of cardiovascular diseases take up a large part of the book. Diseases of the heart and of the blood vessels, together with hypertension, are extensively reflected in cerebral disturbance. The types of brain damage associated with arteriosclerosis, syphilis, and other arterial diseases are discussed in correlation with specific cases and clinicopathological considerations. Reversible changes, specific infections, morphologic changes on a functional basis, all are discussed. Other chapters deal with cerebral reactions to chemotherapy and exogenous intoxicants, as well as with the cerebral manifestations in blood dyscrasias, lung diseases, and liver diseases. There is also a chapter on polyneuritis and neuritis.

The intentions of the book, with its emphasis on correlations, is very praiseworthy. But the effective result is rather unsatisfactory. In the first place the author is not a pathologist. There is an adequate degree of competence when he speaks of morphologic changes strictly of the brain. But a lack of familiarity with general pathology is betrayed whenever he passes beyond his narrow specialty. Possibly the most glaring example is the discussion of kernicterus, where the present day concepts of erythroblastosis, with Rh sensitivity, are not as much as mentioned. For this subject, the most recent listed reference is 1935. Or, similarly, discussions of periarteritis nodosa, general arteriosclerosis, tuberculosis, blood disorders are, from the pathologist's point of view, thoroughly unsatisfactory. In spite of the intent the net result seems to neglect everything except the very narrow domain of pure neuropathology.

Second, the volume gives the impression of hasty preparation, as if the author had collected a certain amount of notes, references, and illustrations, and decided to publish them, regardless of whether the total collection formed a well integrated whole. There is an absence of thoroughness, of careful analysis, and of real integration. The volume is rather an ill-digested hodgepodge of material, the result of which is confusion in the mind of the reader.

If only the author had seen fit to spend another 10 years of unremitting hard work on the preparation of this book, it might have been one of the classics of our time. But as it stands, it can scarcely be recommended.

The illustrations are excellent.

THE MECHANISMS OF CELL DIVISION. By M. J. Kopac and 18 other authors. Annals of the New York Academy of Sciences, Volume 51, Article 8, Pages 1279-1546. Pp. 260, with numerous illustrations. Price $3.50. New York Academy of Sciences, American Museum of Natural History, Central Park W. and 77th St., New York 24, March 23, 1951.

Several currents of research have contributed to a deeper understanding of cell mechanisms: the discovery of the submicroscopic particles by Claude, of the association of many enzymatic activities with mitochondria, fresh insight into the nature of cell organelles, the observation of exchange between nucleus and cytoplasm, the recognition of differences in, and changes in, viscosity of different parts of cells, and investigations of ultrastructure. These themes find

expression, in one form or another, in most of the contributions of this symposium, which deals with one of the profoundest aspects of behavior at this level—cell division. Tissue culture, microsurgery, and centrifugation represent the principal tools of these advances.

On the question of experimental control of cell division, there are contributions concerning the action of agents as diverse as colchicine, nitrogen mustards, podophyllin, substituted phenols, and irradiation. Perhaps of outstanding interest are the series of experiments described by M. Levine wherein rapidly growing cells brought to metaphase by colchicine are exposed, at this most sensitive period, to radiation.

The introductory remarks by E. G. Conklin should on no account be missed.

ELECTROENCEPHALOGRAPHY IN CLINICAL PRACTICE. By Robert S. Schwab, M.D., director of the Brain Wave Laboratory, Massachusetts General Hospital, and associate in neurology, Harvard Medical School. Pp. 195, with 106 illustrations. Price $6.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C.2, 1951.

DIE FRÜHDIAGNOSE DES UTERUSCARCINOMAS: UNTER BESONDERER BERÜCKSICHTIGUNG DES SCHEIDENABSTRICHVERFAHRENS NACH PAPANICOLAOU. By Prof. Dr. Med. Hans Limburg, Universitäts-Frauenklinik Hamburg. Pp. 158, with 75 illustrations. Price $3.50. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O (American zone); Grune & Stratton, Inc., 381 Fourth Ave., New York, 16, 1950.

THE GROWTH, REPLACEMENT, AND TYPES OF HAIR. By J. B. Hamilton, A. E. Light, and 24 other authors. Annals of the New York Academy of Sciences, Volume 53, Article 3, Pages 461-752. Pp. 290, with numerous illustrations. Price $4. New York Academy of Sciences, American Museum of Natural History, Central Park W. and 77th St., New York 24, March 27, 1951.

A TEXTBOOK OF MEDICINE. Edited by Russell L. Cecil, M.D., Sc.D., professor of clinical medicine emeritus, Cornell University, New York, and Robert F. Loeb, M.D., Bard professor of medicine, Columbia University, New York. Eighth edition. Pp. 1627, with 204 illustrations, and 40 tables. Price $12. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C.2, 1951.

THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY. By W. A. Newman Dorland, M.D., Lieutenant-Colonel, M. R. C., United States Army; former member of the Committee on Nomenclature and Classification of Diseases of the American Medical Association. Twenty-second edition. Pp. 1736, with 720 illustrations, including 48 plates. Price $10. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London W.C.2, 1951.

THE 1950 YEAR BOOK OF PATHOLOGY AND CLINICAL PATHOLOGY (JANUARY-DECEMBER, 1950): Pathology. Edited by Howard T. Karsner, M.D., LL.D., medical research advisor to the Surgeon General, United States Navy. Clinical Pathology. Edited by Arthur Hawley Sanford, M.D., professor of clinical pathology, University of Minnesota (The Mayo Foundation); emeritus consultant, Division of Clinical Laboratories, Mayo Clinic. Pp. 455, with 156 illustrations. Price $5. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1951.

HANDBOOK OF MEDICAL MANAGEMENT. By Milton Chatton, M.D., instructor in medicine, University of California Medical School, San Francisco; Sheldon Margen, M.D., clinical instructor in medicine, University of California Medical School, San Francisco, and Henry D. Brainerd, M.D., assistant clinical professor of medicine and pediatrics, University of California Medical School, San Francisco, assistant clinical professor of pediatrics, Stanford University School of Medicine, physician in charge, Isolation Division, San Francisco Hospital. Pp. 508. Price $3. University Medical Publishers, Palo Alto, Calif., 1951.

MEDICINAL CHEMISTRY. Volume (E. S.) I. C. M. Suter, Editor-in-Chief. A Series of Reviews Prepared Under the Auspices of the Division of Medicinal Chemistry of the American Chemical Society. Pp. 473, with numerous tables. Price $12. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, 1951.

This particular book represents a definite need in that we have, for the first time within one volume, a comprehensive treatise on the relationship of chemical structure to the pharmacological action of six different types of compounds. The authors are all authorities in their respective fields, and the book will be particularly useful to chemists active in the synthesis of new medicinal compounds, pharmacologists and biochemists, and to those few physicians who are interested in basic chemical research.

TRANSACTIONS OF THE AMERICAN GOITER ASSOCIATION, ANNUAL SESSION MARCH 9, 10 AND 11, 1950. Shamrock Hotel, Houston, Texas. Pp. 445, with numerous illustrations. Price $11.50. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1951.

The American Goiter Association includes in its membership, and attracts to its annual meetings, a cross section of physicians who devote a considerable part of their efforts to the study of the function and the structure of the thyroid gland. Thus it is to be expected that over the years the annual transactions of this society have documented the evolution of our knowledge of the normal and the pathological thyroid.

The transactions of the 1950 meeting are no exception. Included are reports dealing with diagnostic methods in the study of human thyroid disease, radioiodine in the treatment of hyperthyroidism, toxic effects of radioactive iodine, the adrenals and thyroid disease, exophthalmos, cancer of the thyroid, nontoxic (sporadic) or endemic goiter, and individual papers as follows: "Iodine Compounds in the Blood and Urine of Man," "The Present Status of Surgery in the Treatment of Hyperthyroidism," "A New Intra-Laryngeal Approach for Arytenoidectomy in the Treatment of Bilateral Adductor Vocal Cord Paralysis," "Some Surgical Problems of Nontoxic Goiter and Thyroglossal Tract Lesions."

While it is to be noted that a few manuscripts were not available for publication and that many are published in the *Journal of Endocrinology*, the published record of the transactions do include the discussions that constitute an integral and important part of each of the articles. Indeed, many of the pearls are hidden in fine print. For example, Dr. Alexander Albert's discussion of the article on the adrenals and thyroid disease is a searching critique of methods and interpretations and has a broad application to biological research.

It is impossible to select any individual paper as particularly outstanding, for each is a careful and critical manuscript prepared for judgment by the author's peers. The transactions constitute an integration of the varied approaches and meeting of minds of clinicians and experimentalists in the study of the thyroid, and represent the vanguard of knowledge and investigation in this field.

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LEHRBUCH DER RÖNTGENDIAGNOSTIK. By H. R. Schinz, W. E. Baensch, E. Friedl and E. Uehlinger. Fifth completely revised and enlarged edition. Pp. 528, with 732 illustrations. Price $15.70. Georg Thieme, Diemershaldenstrasse 47 (14a) Stuttgart-O (American zone); Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

SYLLABUS OF HUMAN NEOPLASMS. By R. M. Mulligan, M.D., professor of pathology in the University of Colorado School of Medicine. Pp. 317, with 230 illustrations. Price $7.50. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1951.

This text on human neoplasms covers the subject in a well organized and thorough manner. Within its 288 pages of reading material, it accomplishes in clear-cut fashion that which has been achieved by much larger and more expensive textbooks on tumors. The subject matter is dealt with in 11 chapters on the basis of the cell or the tissue of origin rather than on that of tumors of the various anatomical systems. Clinical findings are presented briefly, and the gross and microscopic features of each tumor are fully and clearly described. There are 230 well chosen and neatly reproduced photomicrographs to illustrate the more important lesions. There are no photographs of clinical or gross material, but that is perhaps a worth-while sacrifice

in reducing the size and the cost of this volume. There is an ample bibliography at the end of each chapter. The articles cited, however, are almost entirely from the American medical literature.

The author has included the derivation of some of the terms employed in the text—a commendable feature—but one would have wished that he had been consistent enough to carry this through for all the more important terms. He seems to have done so through the first 100 pages but to have lapsed thereafter. The supplement, which includes descriptions of benign chondroblastoma of bone, osteoid osteoma, carcinoma of the anus, lymphoid polyp of the rectum and anus, cyst of the pancreas, and cystadenoma of the pancreas, would appear to have been an after-thought. It is hoped that the author will include these in their proper places within the text in a subsequent edition.

This reasonably priced volume is packed full of information and is highly recommended for use by student and postgraduate.

Die Tuberkulose: Ihre Erkennung und Behandlung. By various authors and edited by Prof. Dr. Hellmuth Deist and Prof. Dr. Hermann Krauss. Pp. 760, with 297 illustrations, 20 in color. Price 68 German marks. Ferdinand Enke, Hasenbergsteige 3, Stuttgart, W, 1951.

Leprosy. By E. Grunberg and 19 other authors. Annals of the New York Academy of Sciences, Volume 54, Article 1, pages 1-142. Pp. 142, with numerous illustrations. Price $2.75. New York Academy of Sciences, American Museum of Natural History, Central Park W. and 77th St., New York 24, March 30, 1951.

Biological Actions of the Adenine Nucleotides. By H. N. Green, M.D., M.Sc., professor of pathology, University of Sheffield, and H. B. Stoner, M.D., B.Sc., research assistant, University of Sheffield, Department of Pathology. With a foreword by Sir Edward Mellanby, G.B.E., K.C.B., M.D., SC.D. Pp. 221, with 65 illustrations and 36 tables. Price 25 shillings net. H. K. Lewis & Co., Ltd., 136 Gower St., London, W.C. 1, 1950.

This monograph presents an account of fundamental contributions made over the last 10 years by the authors and their associates to the biochemistry of the adenine nucleotides. The pharmacological effects of these compounds on the principal body systems are examined in great detail, and for these actions they have provided a valuable and original text. Nevertheless, this presentation embodies a peculiar ambivalence, arising from the fact that this work originally began as an investigation of shock and of the role in shock of the tissue elements, the local or the metabolic factors. It was, in fact, early shown that much of the shock-inducing effect of fresh muscle extract, for example, could be attributed to its content of adenosine triphosphate, and from this the authors were led to consider the role of purine compounds in the pathogenesis of shock. Painstaking experimentation conducted over several years seems to have failed to bridge the gap between nucleotide and traumatic shocks; the main advance in this field of knowledge has therefore been along the lines of pharmacological effects, as noted above.

A Text-Book of X-Ray Diagnosis. Second edition. Edited by S. Cochrane Shanks, M.D., F.F.R., director, X-ray diagnostic department, University College Hospital, London, and Peter Kerley, M.D., F.F.R., D.M.R.E., director, X-ray department, Westminster Hospital; radiologist, Royal Chest Hospital, London. Volume I. Pp. 434, with 439 illustrations. Price $12. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

This volume is designed primarily as a supplement to the previous edition, bringing the text up to date, particularly on such subjects as cerebral angiography and ventriculography. The chapters dealing with the teeth and jaws, and the eye, give valuable information on these often neglected aspects of roentgenology. The subject matter is complete without detailed emphasis on the rarer lesions, with adequate coverage of basic anatomy, physiology, and technique. The text is profusely illustrated with well-chosen diagrams and roentgenograms.

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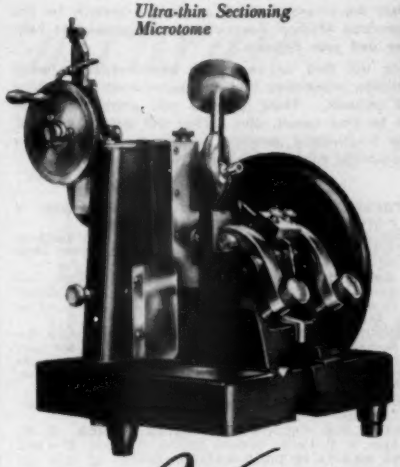

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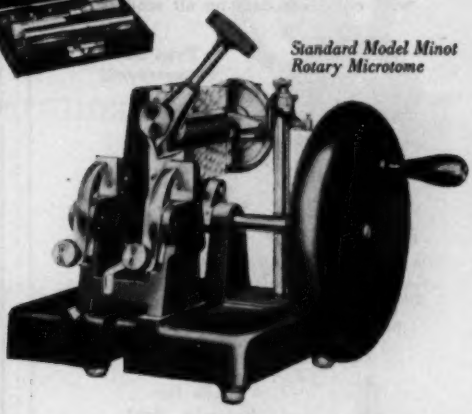

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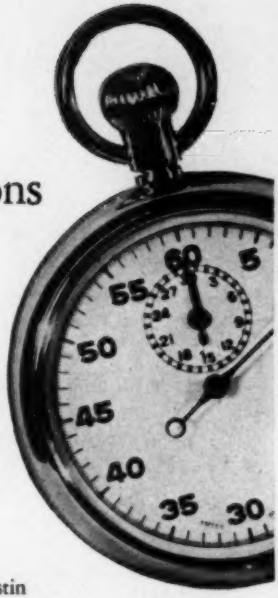

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1. Shapiro, S., Weiner, M., et al.: Am. Heart J. **40**: 766 (Nov.) 1950

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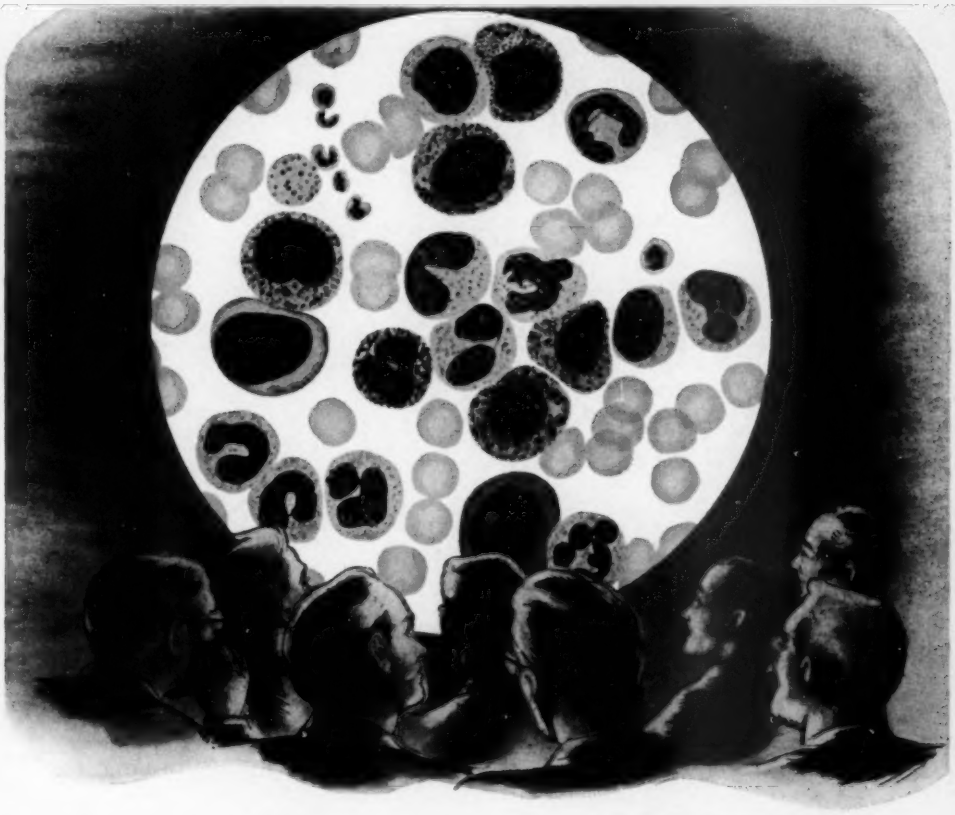

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